

Fall 1980

THE INFLUENCE OF FULVIC ACID ON  
COPPER(II), CADMIUM(II) AND ZINC(II)  
REMOVAL FROM DRINKING WATER BY  
ALUM COAGULATION AND COPPER(II)  
AND CADMIUM(II) COMPLEXING  
CAPACITY MEASUREMENTS OF FULVIC  
ACID AND NATURAL FRESHWATER  
SAMPLES BY DIALYSIS TITRATION

RALPH EDWARD TRUITT

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University of New Hampshire

PH.D.

1980

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THE INFLUENCE OF FULVIC ACID ON  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  AND  $\text{Zn}^{2+}$   
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DIALYSIS TITRATION

BY

Ralph Edward Truitt  
B.A., University of Massachusetts, 1975

DISSERTATION

Submitted to the University of New Hampshire  
in Partial Fulfillment of  
the Requirements for the Degree of

Doctor of Philosophy  
in  
Chemistry

September, 1980

This dissertation has been examined and approved.

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JULY 1 1980

Date

To my wife, Debby

## ACKNOWLEDGEMENTS

I am grateful to several people for their contributions to this research, but two people in particular. My thanks to Dr. James H. Weber for his support and encouragement throughout the entire project and for providing funding from his Office of Water Resources Technology Grants B004-NH and A046-NH, which were administered by the Water Resources Research Center at the University of New Hampshire. My gratitude to Dr. C.L. Grant for invaluable instruction and advice on experimental design and statistics.

My gratitude also to my wife, Debby, for her considerable help in preparing this manuscript and for her patience and support over the years. Dr. James H. Weber and Dr. C.L. Grant have my thanks for reading this manuscript and suggesting improvements and corrections.

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## ABSTRACT

THE INFLUENCE OF FULVIC ACID ON  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  AND  $\text{Zn}^{2+}$   
REMOVAL FROM DRINKING WATER BY ALUM COAGULATION  
AND  $\text{Cu}^{2+}$  AND  $\text{Cd}^{2+}$  COMPLEXING CAPACITY MEASUREMENTS  
OF FULVIC ACID AND NATURAL FRESHWATER SAMPLES BY  
DIALYSIS TITRATION

by

RALPH EDWARD TRUITT

University of New Hampshire, September, 1980

The metal ion complexing chemistry of organic ligands commonly found in drinking water sources is the object of the studies in this dissertation. These ligands, called humics as a class, were modelled in some cases by a soil-derived humic material called fulvic acid. The role of natural humics in the transport and speciation of dissolved metal ions in drinking water sources, particularly health hazards like  $\text{Cd}^{2+}$ , is investigated. Some properties of soil-derived fulvic acid (SFA) were also elucidated.

One study modelled a drinking water clarification and decolorizing process using aluminum hydroxide coagulation to measure  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  removal from solution by the coagulation treatment. The effect of SFA on these metal ion removals was determined in a statistically designed

experiment where the relative effects of changing metal ion concentration,  $\text{Al}^{3+}$  concentration, solution pH, and SFA concentration on metal ion removal were revealed. Over the variable levels used in the experiment, increasing pH from 5 to 7, and increasing  $\text{Al}^{3+}$  dosage caused the greatest overall increase in  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  removal. Fulvic acid, representing naturally-occurring humics, aided metal ion removal when enough aluminum coagulant was used to remove all dissolved fulvic acid. When insufficient coagulant was used and some fulvic acid remained in solution, the humic material stabilized dissolved metal ions by formation of a soluble metal ion complex and resolubilized metal ions ordinarily coprecipitated with the aluminum hydroxide floc.  $\text{Cu}^{2+}$  is very effectively removed on average (>90%) under the experimental conditions, but  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  are not (<20%).

To perform the metal ion removal experiment, and to filter natural water samples with no substantial metal ion losses due to wall effects or contaminations, a microfilter apparatus was assembled and evaluated. The apparatus has all plastic surfaces for solution contact and allows vacuum filtration directly into a polypropylene receiving vessel, thereby eliminating the need for a filter flask.  $\text{Cu}^{2+}$  losses and contaminations during filtration of standard  $\text{Cu}^{2+}$  solutions through the plastic filter apparatus or a glass filter support, with cellulose acetate or polycarbonate filter membranes, were measured in a statistically



designed experiment. The results show small  $\text{Cu}^{2+}$  losses with the plastic filter support and either membrane (0-13% losses) compared to the glass support with either membrane (25-79% losses). The use of an all plastic filter apparatus for the filtration of natural water samples is recommended to minimize changes to the native levels of dissolved metal ions.

A dialysis separation technique, where complexed and unbound metal ions are distinguished in the determination of natural freshwater metal ion binding capacities, was evaluated in another study. The dialysis technique metal ion binding results could not be distinguished from potentiometric titration results in a statistical comparison of the two methods. Nomenclature to describe metal ion binding in natural water systems was suggested. A brief literature survey of metal ion binding studies was described. The dialysis technique was applied to the measurement of 6.25  $\mu\text{M}$  EDTA, 15.5  $\mu\text{M}$  SFA and seven natural water sample capacities of  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  complexation. The metal ion binding capacities of SFA increased with pH and  $\text{Cu}^{2+}$ -SFA capacities were greater than  $\text{Cd}^{2+}$ -SFA capacities at the same pH. The  $\text{Cu}^{2+}$  binding ability of the natural water samples correlates negatively with their alkalinity, pH, hardness and conductance.  $\text{Cd}^{2+}$  binding capacities did not correlate well with any water sample characteristic.

## CHAPTER 1

### THE SIGNIFICANT PROPERTIES OF HUMIC MATERIALS IN NATURAL WATER SYSTEMS

#### Introduction

The purpose of this introductory chapter is to briefly define and describe a class of organic compounds found in nature whose properties are the subject of this dissertation. These organic compounds, called humics, have undergone much study, particularly in the last 25 years, but investigators have yet to produce a clear, universal, unambiguous description of humics. In fact, due to the chemical complexity of this material and the lack of standard isolation and analysis procedures, much of the information currently available is contradictory or not comparable.

There is much information still needed about the chemistry of humics in the natural environment since many areas of research into the chemical behavior of humic matter are unexplored and the information available from previous work is often incomplete. My intended contribution to this area of investigation, aside from providing more data on humic matter properties, is one of methodology. The work described in this thesis approaches the study of humic material chemistry with a three-fold theme. First, I have tried to impose order on experimental design. Statistical experi-

mental design is invaluable for the study of such a complex matrix as natural fresh waters or ill-defined materials like humics where there is always a great probability of having uncontrolled experimental variables. The second theme of the work in this thesis is method evaluation. With the measurement of certain properties of humics in drinking water, an effort is also made in this work to critically evaluate the application of conventional analytical techniques to the study of natural freshwater chemistry or to the study of humic material chemistry. It is a perverse aspect of the analysis of natural systems (or humic materials) that there are rarely simple or convenient procedures that may be applied without interferences or difficulties. All the procedures and techniques used in this study are therefore subject to some scrutiny. The third goal of this study is to provide more information on humic matter properties.

In this introduction only the important properties of some specific humic compounds will be emphasized. For more information, several review articles and books that have discussed the general nature of humics in great detail are available (Jackson, Jonasson & Skippen, 1978; Jackson, 1975; Mantoura & Riley, 1975; Flaig, Beutelspacher & Reitz, 1975; Singer, 1973; Stevenson & Butler, 1969; Schnitzer & Kahn, 1972; Gamble & Schnitzer, 1973). Some properties of humic matter reviewed in these sources will not be discussed here.

### Description of Humic Materials

Humic materials are the most common natural products on earth. They are ubiquitous: in varying amounts these materials are found in the marine environment and in most soils and freshwaters. Humic compounds, formed by the natural degradation of plant and animal products, are complex mixtures of compounds rather than discrete molecules. While this greatly complicates analyses and causes ambiguity in some of the properties of the material, average characteristics can still be determined. The relative stability of these materials to further degradation or other reactions accounts for their prevalence over non-humic organic compounds in the environment like amino acids, proteins, carbohydrates, etc.

It is not only for their environmental prevalence that humic compounds are the subject of chemical analysis. These substances have significant roles in the speciation, transport and bioavailability of both nutrients and pollutants in soils and natural waters (Reuter & Perdue, 1977; Davis & Leckie, 1978; Wagemann & Barica, 1979; Jackson & Skippen, 1978; Szilágyi, 1974; Langford et al., 1977). In particular, they are implicated in the retention of insecticides in soils and they are the source of haloforms when some drinking waters are disinfected by chlorination (Rook, 1977; Choi & Chen, 1976). Some of the  $K^+$ ,  $Na^+$ , nitrate, phosphate and other inorganic plant nutrients in soils are associated with clay adsorbed humic compounds, which influences the

rate these nutrients leach from the soil (Schnitzer, 1971). Most important with respect to this thesis, humics have also demonstrated metal binding ability. It is this property that is of interest here, particularly since humic matter binding of metal ions influences the occurrence of undesirable metal ions in drinking water (Truitt, 1975).

Humic substances, the more stable intermediates or final products of natural decomposition processes, are structurally related to lignites and coals. Unfortunately, structural information obtained by many investigators has not yet produced a clear model of a humic compound molecule. Considering that humics are a complicated mixture of molecules in various stages of polymerization, attempting to structurally identify an average humic molecule may have no meaning. Nonetheless, from functional group analysis, elemental analysis and molecular weight studies, various structures have been drawn (Schnitzer & Kahn, 1972; Jackson et al., 1978; Stevenson & Butler, 1969). These structures emphasize aromaticity, functionalities such as carboxyl, hydroxyl, carbonyl and methoxy groups, and polymerization with hydrogen bond linkages.

The humic matter nomenclature originates with agricultural scientists over a century ago (Flaig et al., 1975) and is essentially unchanged in modern usage. The entire group of materials described as humics are divided into several fractions based upon their solubility in aqueous and alcoholic solution (Figure 1). A simple classification scheme

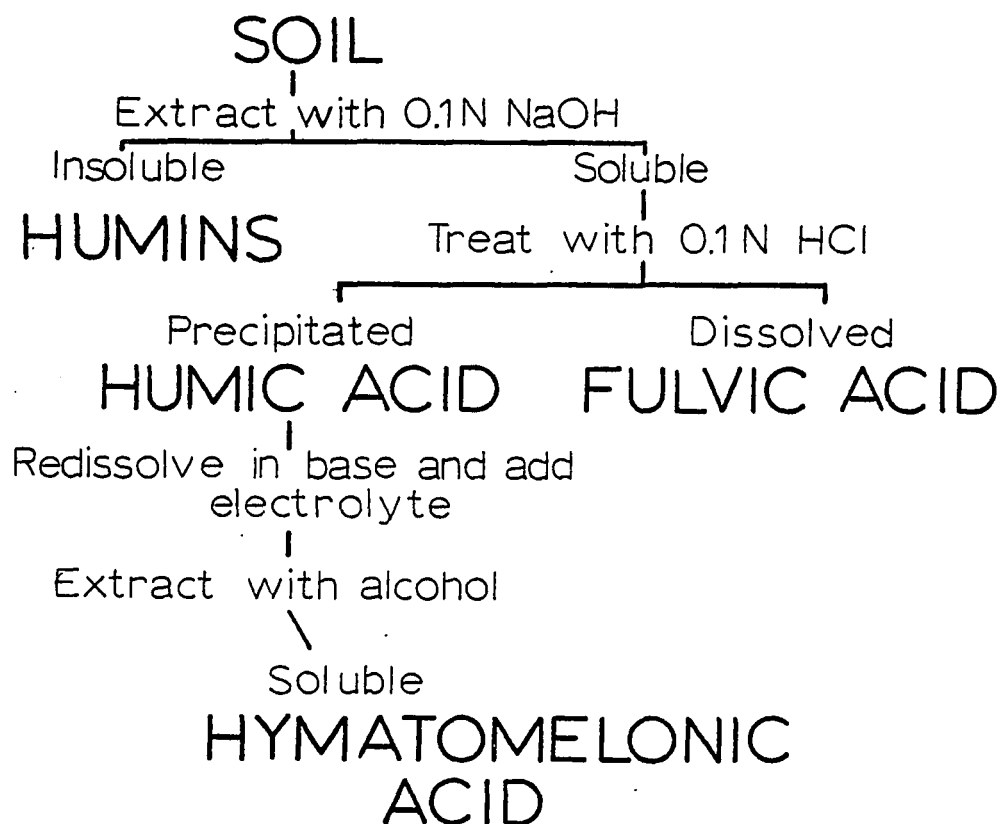


Figure 1. Extraction Procedure and Nomenclature of Soil Humic Material Fractions (Schnitzer & Kahn, 1972)

is generally used in modern humic matter studies, which classifies all humics as fulvic acid (FA), humic acid (HA), or humin. These groups are distinguished by their aqueous solubility: humins are insoluble in water at any pH, humic acids precipitate from acidic aqueous solution, and fulvic acids are soluble at any pH. In this study, a humic fraction soluble at pH 1 extracted from soil will be used as a model of naturally occurring organic matter. This material is called soil fulvic acid (SFA) and has been characterized by Wilson and Weber (1979a, 1979b), Weber and Wilson (1975), Saar and Weber (1979), Bresnahan et al. (1978), and Truitt and Weber (1979a, 1979b, 1979c).

#### Soil Fulvic Acid (SFA) Properties

Data obtained by these investigators are summarized in Tables 1 and 2. SFA may be described as typical fulvic acid material compared to other isolated materials (Schnitzer & Kahn, 1972). Wilson and Weber (1977a) report a number average molecular weight of 644. Elemental composition analysis reveals that SFA is 53% carbon, 3% hydrogen, 1% nitrogen, 42% oxygen (by difference, Table 1) and 1% ash.

Some solution phase behavior of SFA pertains to the experiments detailed in this thesis. For instance, humic materials have some surface active properties in solution (Davis & Leckie, 1978). In particular their mercury surface activity is an analytical impediment as discussed in Chapter 4 (Buffle et al., 1978; Greter et al., 1979).

SFA has some important pH and ionic strength dependent

Table 1. Soil Fulvic Acid Properties

Elemental Analysis (%)<sup>a</sup>

C	53.1
H	3.24
N	0.90
Ash	0.8

Functional Groups (mequiv./g)<sup>a</sup>

Carboxyl	8.2
Phenolic OH	5.2
Carbonyl	3.5
Total Acidity	13.4

Molecular Weight<sup>b</sup> 644<sup>a</sup>Weber & Wilson (1975)<sup>b</sup>Wilson & Weber (1977)



Table 2. SFA Metal Binding Constants<sup>a</sup>Cadmium<sup>a</sup>

<u>pH</u>	<u>K(x10<sup>-3</sup>)</u>	<u>[SFA] (x10<sup>-4</sup> M, pH 6)</u>	<u>K(x10<sup>-3</sup>)</u>
4.0	1.7	0.3	29
5.0	6.3	0.6	24
6.0	12	1.3	18
7.0	21	2.4	13
8.0	43	4.1	14
		5.6	12

Copper<sup>b</sup>

<u>pH</u>	<u>K<sub>1</sub> (x10<sup>-5</sup>)</u>	<u>K<sub>2</sub> (x10<sup>-5</sup>)</u>
4.0	4	0.09
5.0	10.0	0.12
6.0	20.0	0.06

Lead<sup>a</sup>

<u>pH</u>	<u>K(x10<sup>-4</sup>)</u>
4.0	1.0
4.5	2.0
5.0	7.9
6.0	200

<sup>a</sup>Saar & Weber (1979)<sup>b</sup>Bresnahan et al. (1978)

chemical and physical behavior. To begin with, humics exhibit polymer size and possibly configuration dependence on pH and ionic strength (Gjessing, 1971; Lindquist, 1974; Swift & Posner, 1971). As pH increases or ionic strength decreases, the molecule becomes smaller, possibly through a coiling of the polymer. At the same time more anionic sites are produced on the polymer by increased acid neutralization or dissociation. This size and charge alteration has significant effects in the dialysis experiments described in Chapter 4.

The other important pH and ionic strength dependent property of SFA is its metal chelating ability. The metal binding properties of fulvic acid have been assigned to salicylate, phthalate and carboxylic functionalities by other investigators (Jackson, Jonasson & Skippen, 1978; Stevenson, 1977; Gamble et al., 1970). Weber and Wilson (1975) report total acidity of 13.4 mequiv/g, mainly attributed to carboxyl and phenolic hydroxyl functionalities (Table 1). Saar and Weber (1979) and Bresnahan et al. (1978) have measured conditional metal-SFA binding constants by ion selective electrode potentiometry. These constants, given in Table 2, agree with titration results of other humic materials which indicate that fulvic acid complex stabilities follow the general order  $\text{Cu}^{2+} = \text{Pb}^{2+} > \text{Cd}^{2+} = \text{Zn}^{2+}$ . The stoichiometry of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  fulvate binding is dependent on the [metal ion]/[SFA] ratio, pH, and on the metal ion involved. As a result, metal ion bound/ligand ratios are often ambi-

guous. In view of its metal binding ability, the transport of metal ions in fresh waters by complexation with dissolved organic matter is a mechanism requiring elucidation since hazardous or otherwise unwanted metals can ultimately arrive in drinking water in association with dissolved fulvic acid.

## CHAPTER 2

# THE INFLUENCE OF FULVIC ACID ON THE REMOVAL OF TRACE CONCENTRATIONS OF $\text{Cd}^{2+}$ , $\text{Cu}^{2+}$ AND $\text{Zn}^{2+}$ FROM WATER BY ALUM COAGULATION

### Introduction

Many drinking water sources, and some treated waters, contain undesirable metal ions. The presence of some ions is a matter of concern, especially where the dissolved metal ion concentrations in treated water exceed maximum levels allowed by regulatory agencies (Méranger et al., 1979; McCabe, 1974). Metal ions are undesirable because they are public health hazards (e.g.,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Hg}^{2+}$ ), or they cause aesthetic problems with water taste and staining (e.g.,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ), even at  $\mu\text{g}$  to  $\text{mg/L}$  concentrations (Craun & McCabe, 1975).

The presence of these ions in drinking water sources is a problem because drinking water treatment processes specifically designed to remove trace metals are not common. However, the use of coagulants is a typical water treatment procedure generally aimed at the removal of suspended matter, dissolved organic matter and inorganic species that contribute to water hardness. Coagulation processes incidentally cause the partial removal of trace metal ions from waste water (Linstedt, Houck & O'Connor, 1971; Nilsson, 1971;

Oliver & Cosgrove, 1974; Maruyama, Hannah & Cohen, 1975; Leentvaar, Buning & Koppers, 1978) and drinking water supplies (Logsdon & Symons, 1973; O'Connor, 1974; Naylor & Dague, 1975). The coagulants, usually hydrolyzed aluminum sulfate (alum) or ferric chloride, act to remove metal ions from solution by coprecipitating or adsorbing the ions during the flocculation process.

While the ability of these coagulants to remove dissolved metal ions from solution has only been suggested, the fate of dissolved organic matter, especially humic materials, in the coagulation processes is well documented. Van Bree-men et al. (1979) quantified fulvic acid removal as a function of pH and alum dosage in jar studies modeling water treatment processes. They found maximum solution color and total organic carbon (TOC) removal to occur in a pH range of 5 to 7. This corresponds to the pH region of lowest aluminum hydroxide solubility. Fulvic acid removal was also a function of alum concentration. Substantial (>80%) humic acid removal by alum coagulation in this pH range was reported by Edzwald et al. (1977), who studied the influence of electrolytes on color removal efficiency. Humics were found in tap water despite water treatment processes however (Farrah et al., 1978).

The influence of humic materials on the metal removal by a typical water treatment coagulation process is not reported. Considering that humic compounds are both ubiquitous and metal coordinating compounds, their influence on

the transport of metal ions through water treatment processes is probably great. The role of these metal complexing agents has been largely overlooked and the mode of metal ion removal by coagulation processes with humic matter present has not been demonstrated.

In this water treatment study, the extent of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  removal by alum coagulation was determined as a function of metal ion concentration, coagulant concentration, pH and fulvic acid concentration. The experiments were performed to model drinking water treatment processes as much as possible so that the results could be related to treatment plant conditions. In three ways this study is different from previous work. First, introducing fulvic acid as a natural organic matter model will elucidate the effect of these natural metal ion complexing agents on metal removal during a typical drinking water treatment. Second, by performing the study in a factorial experimental design and evaluating the data by analysis of variance (ANOVA), the relative amounts of metal removal due to coprecipitation or adsorption on the alum floc, due to hydrolysis of the analyte metal ions, and/or due to some mechanism involving complexation with fulvic acid can be distinguished. In addition to being able to assign the relative influence of each metal removal mechanism, the statistical experimental design has one more benefit: synergistic or antagonistic interactions of the experimental variables can be distinguished. Interactions such as these, i.e., some metal ion removal ability of

variables acting in concert that is greater or less than the sum of the variables acting independently, can only be revealed by ANOVA of a designed experiment. The third unique feature of this study is that considerable attention has been given to the manipulation of trace metal solutions to avoid analytical errors. Specifically, metal ion contaminations and losses during filtration of trace metal ion solutions at pH 5 to 8 are determined, and a device to minimize these problems was evaluated. These filtration experiments are the subject of the next chapter.

#### Factorial Experimental Design and ANOVA

Since the statistical design is critical to the results of the water treatment experiment, the procedure and terminology will be detailed.

Factorial design of the experiments enables one to simultaneously investigate the effects of all four independent variables on metal ion losses. Since there are four variables to be considered,  $2^4$  experiments are necessary to measure the effect of all combinations of variables when each variable is tested at a high and a low level. Center point experiments, where variable levels are the average of high and low levels, are added to the design to detect any non-linearity in the experimental response between the extreme levels. The results of all the experiments are statistically analyzed by analysis of variance (ANOVA).

The first requirement in establishing a factorial experimental design is to decide what variables are to be

tested. In this study, where metal ion removal during a water treatment process with humic materials present is studied, the effects of varying metal ion concentration, pH, alum concentration, and soil fulvic acid concentration are determined. Next, a high and low level are assigned to each variable. These variable settings should be chosen to adequately cover a range normally encountered for the variables. Variable levels in this study were chosen to cover a range of values normally seen by water treatment plants, except for metal ion concentrations which were chosen to be within the detection limit of flame atomic absorption spectroscopy (AAS) and to be high enough to allow filtration with small background losses. Once variable levels are established, the high level, center point and low level are designated at +, 0 and -, respectively. Replication of experiments provides the experimental random error term needed for analysis of variance. Randomization of the experimental order is needed to avoid systematic errors such as aging of stock solutions.

Table 3 has an example of a factorial experimental design. This design was used to establish metal ion losses in the water treatment experiment procedure. In this design there are  $2^4$  combinations of the four variables, each at high and low settings, and a centerpoint experiment. This type of design is therefore designated a  $2^4+1$  design. The experiments are shown in Table 3 as combinations of +, 0 or - settings of each variable. The experimental order in Table 3 is randomized and in this design the centerpoint is



Table 3. Percent Metal Ion Losses for the  $2^4 + 1$  Factorial Experiment

					Level		
					+	0	-
A = [Metal ion] ppb					1000	750	500
B = [Alum] ppm					50	30	10
C = pH					7	6	5
D = [SFA] ppm					20	10	0

Experiments					Percent Losses <sup>a</sup>		
No.	A	B	C	D	Cu <sup>2+</sup>	Cd <sup>2+</sup>	Zn <sup>2+</sup>
1	+	+	+	+	93	59	81
2	+	+	+	-	93	5	53
3	+	+	-	+	72	-5	-3
4	+	+	-	-	23	-3	0
5	+	-	+	+	7	0	-7
6	+	-	+	-	54	2	16
7	+	-	-	+	38	-6	-6
8	+	-	-	-	13	-5	-4
9	-	+	+	+	90	32	82
10	-	+	+	-	96	14	49
11	-	+	-	+	32	2	-1
12	-	+	-	-	24	2	0
13	-	-	+	+	6	3	-1
14	-	-	+	-	64	8	21
15	-	-	-	+	14	0	1
16	-	-	-	-	24	-3	-2
17	0	0	0	0	48	5	15
18	0	0	0	0	49	0	10
19	0	0	0	0	51	5	13
20	0	0	0	0	62	8	14
21	0	0	0	0	57	7	15
22	0	0	0	0	b	3	9

<sup>a</sup> A negative loss is an excess<sup>b</sup> Not determined

run six times so random error can be calculated.

When the experiments of the factorial design have been completed, the influence of each of the four experimental variables and the effect of all variable interactions can be calculated by ANOVA. The result of these calculations is the F ratio (or variance ratio), which measures the significance of the effect each variable and each interaction of variables had on metal ion removal from solution, taking experimental random error into account. Here lies the power of factorial design and ANOVA: not only is the effect of each variable isolated from the effects of all the other variables and random error, but synergistic or antagonistic interaction effects between the variables can be identified. These variable interaction effects, which cannot be measured in one variable at-a-time experiments, can provide metal ion removal mechanism clues. A mechanism will be deduced from the results of this water treatment experiment and from what is known about fulvic acid and aluminum solution properties.

### Experimental

#### Procedures

Two sets of experiments were performed. Data were collected from a  $2^4$  factorial experiment in which the design in Table 3 was used, and from blank experiments where the design in Table 3 was used without alum to determine background experimental metal ion losses and SFA losses. Percent metal ion losses from the full factorial experiment was

analyzed by ANOVA.

To prepare each solution defined by the experimental design given in Table 3, the appropriate amounts of SFA, metal ion and alum were added to approximately 47 mL of water in a 50 mL volumetric flask. The pH was adjusted with ca. 0.01 M KOH, then the solution was diluted to 50 mL. The solution was stirred for 5 minutes, then was allowed to stand quiescent for 30 minutes. At the end of this coagulation period, the solution was filtered using an all-plastic apparatus, which is diagrammed, evaluated and described in great detail in Chapter 3. About 25 mL CO<sub>2</sub>-free (pH 7) water was used to rinse the volumetric flask and filter apparatus. The rinsings were collected with the filtrate in a 100 mL volumetric flask and diluted to the mark with 1.0 mL reagent HNO<sub>3</sub>, 1.0 mL 1.00 M KNO<sub>3</sub> and water. Metal ion concentrations were determined by atomic absorption spectrometry (AAS) using a Techtron AA5 spectrometer (Varian Corp., Melbourne, Australia). UV absorption at 260 nm was used to determine SFA concentrations using a Cary 14 spectrophotometer (Applied Physics Corp., Monrovia, California).

Between filtrations the filter apparatus was cleaned by discarding the used membrane, rinsing with 250 mL ca. 0.1 M KOH, rinsing with 250 mL ca. 0.1 M HNO<sub>3</sub>, and finally rinsing with 50 mL water. Blank studies demonstrated that no Cu<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup> or SFA remained after this treatment.

All volumetric flasks were cleaned prior to each experiment by rinsing with water, soaking in 0.1 M KOH, rinsing

Table 4. Summary of Metal Ion Losses in the Water Treatment Experiment

<u>Metal Ion</u>	<u>Range of Removal</u>	<u>Average Removal</u>
Cu <sup>2+</sup>	6-96%	48%
Cd <sup>2+</sup>	0-59%	7%
Zn <sup>2+</sup>	0-82%	18%

with water, soaking in 2 M  $\text{HNO}_3$ , and finally rinsing with water.

## Results and Discussion

### Water Treatment Experimental Results

Metal ion removal. The percent  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  losses measured in all the experiments of the  $2^4+1$  factorial design are given in Table 3. A listing of all the metal losses (Table 4) reveals differences in both the range of percent metal removal and in the average percent metal ion removal among the  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  experiments. Apparently the experimental conditions used in the water treatment experiment were optimal for  $\text{Cu}^{2+}$  removal, but not for  $\text{Cd}^{2+}$  or  $\text{Zn}^{2+}$  removal. In the  $\text{Cu}^{2+}$  experiment, metal ion losses from essentially zero to essentially 100% were effected, and the average loss was 48%. This wide range of losses is desirable because small metal removal effects are more distinct; that is, the experiment is more sensitive. On the other hand,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  losses, which were only 7% and 18% on average, occurred over a smaller range than  $\text{Cu}^{2+}$  losses. In summary,  $\text{Cu}^{2+}$  is much more effectively removed from solution than either  $\text{Cd}^{2+}$  or  $\text{Zn}^{2+}$  under comparable experimental conditions.

Background metal ion losses. In the study of  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  removal by treatment with alum, a first consideration was the extent to which these metals are removed from solution by processes other than alum coagulation. Back-

ground metal ion loss control studies were needed since all three of the metal ions in this study may be lost by adsorption on vessel walls (Tögl, 1972; Struempler, 1973; Beneš & Kopicčka, 1974; King, Rodriguez & Wai, 1974; Batley & Gardner, 1977). Another background effect is the potential formation of insoluble metal hydroxides or metal ion-fulvic acid complexes that will not pass the filter. Finally, metal ion contaminations are possible. Because of the common occurrence of  $\text{Zn}^{2+}$  in laboratory materials and reagents, this metal ion is most susceptible of the three ions to contamination problems (Robertson, 1968), but improperly cleaned vessels or accidental contamination could result in  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  background levels. In the determination of background metal ion losses, all effects just mentioned are combined. Losses were determined during filtration with the polycarbonate and polypropylene filter apparatus under all the water treatment experimental conditions except the presence of alum.

Background water treatment experiment metal ion losses, which were performed in the Table 3 design with no aluminum sulfate present, gave an average cadmium loss of 1.8% ( $\pm 4.0$ ), an average zinc loss of 9.6% ( $\pm 7.3$ ), and an average copper removal of 3.0% ( $\pm 2.4$ ) which does not include a 24% copper loss measured in one experiment. Since no alum coagulation occurs in these background experiments, all the metal ion losses occur because of sample manipulations and container wall adsorption. Sample contaminations by metal ions

are also possible.

The most important conclusion from these experiments is that background losses or contaminations are generally small. There was one exception already noted. The experiment with 1 mg/L  $\text{Cu}^{2+}$ , 0 mg/L SFA and pH 7 resulted in a 24% loss. This copper loss, which is due to the filtration of  $\text{CuO}$ , is predictable from the solubility product constant of this species. Copper and cadmium losses in all the other experiments were small. A hypothesis that the average background metal ion losses are significantly different from zero was tested at the 99% confidence level. A one-tailed t-test of this hypothesis (Natrella, 1963, p. 3-4) indicates that the copper and zinc mean background losses are real (different from zero), but the cadmium losses cannot be distinguished from zero. Zinc losses were larger than copper and cadmium losses which may be attributed to contamination problems and/or the relative insensitivity of the zinc AAS measurements. Both of these mitigating factors are characteristic of trace zinc analyses. That SFA does not interfere with zinc AAS determinations was assured by experiments that showed no discernable zinc determination differences among solutions with 0, 500, 750 and 1000 mg/L SFA concentrations.

The use of these small background metal ion losses as correction factors in the full water treatment experiment (alum present) is not theoretically justified. For one reason, the cadmium background losses are essentially zero. Also, as the full experimental results will show, there is

a strong SFA-alum interaction effect on metal ion losses that is not measured in the alum-free background experiments. This interaction effect will be discussed with the other ANOVA results which all indicate that the behavior of the three filtered metal ions is quite different in the presence of alum. As a result, the small background losses resulting from manipulating each solution are not distinguished from metal ion losses due to alum coagulation in the full water treatment experiment (alum included).

Color removal. Fulvic acid loss and metal ion removal was monitored in the water treatment experiments. Table 5 has a summary of fulvic acid removal in the alum coagulation experiments, as determined by the 260 nm absorbance (color) of the solutions. These average fulvic acid losses undergo a sudden change as a function of alum dosage. With alum dosages greater than 10 mg/L, the alum coagulation process is apparently quite effective in removing the dissolved organic matter, which is its function in drinking water treatments. Indeed, at the high alum dosages, essentially all the fulvic acid was removed. In contrast, virtually none of the fulvic acid was removed with the 10 mg/L alum dosage. In this experiment, equimolar quantities of alum and SFA resulted in little color removal. The consequences of using an alum dosage insufficient to remove any of the fulvic acid will be mentioned in the discussion of the water treatment experiment ANOVA results.



Table 5. Average Percent Color Removal in the Water Treatment Experiments

<u>Expt.</u>	<u>50 mg/L Alum</u>	<u>30 mg/L Alum</u>	<u>10 mg/L Alum</u>
Cu	95	98	0.3
Cd	95	91	2
Zn	94	91	3

### ANOVA Results

Prior to a discussion of what effect each experimental variable and variable interaction had on copper, cadmium and zinc removal, the data can be examined by analysis of variance (ANOVA) to determine which effects are significant compared to random error. While the actual calculation and ANOVA table are found in Appendix 1, Table 6 lists the effects and F ratios of all the variables and interactions for the copper, cadmium and zinc experiments.

In Table 6 the underlined F ratios identify those variables and variable interactions that significantly affected metal ion removal in the water treatment experiment. There is a 99% confidence that the effect of these significant factors is not due to random experimental error. The statistics indicate significant factors by locating relatively large differences in metal ion removal when the variable level is changed from its low to its high setting. If metal ion losses are a strong function of the variable setting, and this effect is demonstrably different from the effect of random error, the variable is identified as significant in its effect on metal ion removal.

Briefly reviewing the F ratios in Table 6, ANOVA reveals that pH (C) and alum concentration (B) dominate the overall metal ion removal in all three experiments. Fulvic acid concentration (D) is significant in the removal of cadmium, but not copper or zinc. Some interactions of the four experimental variables had significant metal ion removal

Table 6. Water Treatment Experiment Variable Effects and F Ratios

Factor <sup>a</sup>	Effects <sup>b</sup> (% removal)			F Ratios <sup>c</sup>		
	Cu	Cd	Zn	Cu	Cd	Zn
A	5	-1	-2	3	1	3
B	38	13	30	<u>163</u>	<u>87</u>	<u>554</u>
C	33	18	39	<u>122</u>	<u>151</u>	<u>895</u>
D	-5	9	2	<u>3</u>	<u>32</u>	<u>2</u>
AB	4	3	0	2	4	4
AC	-8	4	0	7	6	0.1
AD	12	5	-2	15	8	2
BC	22	10	29	<u>57</u>	<u>57</u>	<u>492</u>
BD	18	11	13	<u>35</u>	<u>43</u>	<u>96</u>
CD	-23	8	2	<u>60</u>	<u>32</u>	<u>3</u>
ABC	-2	4	1	0.5	7	0.5
ABD	6	5	-1	0	9	0
ACD	-7	6	0	6	15	0
BCD	7	10	14	6	<u>52</u>	<u>116</u>
ABCD	-1	4	-1	0.2	<u>7</u>	<u>0.5</u>

<sup>a</sup>Factors A,B,C and D are metal ion concentration, alum concentration, pH and SFA concentration, respectively.

<sup>b</sup>Values are rounded to whole numbers.

<sup>c</sup>Underlined values indicate 99% confidence level significance. Critical F values for 99% confidence level are 21.20 for the Cu experiments and 16.26 for the Cd and Zn experiments.

effects. Interactions between pH and alum concentration (BC), and between alum concentration and SFA concentration are important in all three water treatment experiments. Only zinc removal is not significantly changed by a pH and SFA concentration interaction (CD). Both zinc and cadmium experiments have a significant three-variable interaction between pH, alum and SFA levels (BCD). These three variable effects, which are unlikely, probably reflect the strong B, C and BC effects.

Identifying the ineffectual variables is also interesting. In Table 6, metal ion concentration (A) and all interactions with this variable have non-significant F ratios. Clearly, changing metal ion levels from 500 ppb to 1000 ppb made no difference in copper, cadmium and zinc removal that could be confidently distinguished from experimental error. These metal ion levels are quite high, as previously mentioned, so the removal efficiency of the water treatment process is not different for metal ion concentrations of 500 ppb or larger. On the other hand, the overall non-significance of SFA concentration changes (D) is unexpected considering the SFA removal in the experiments (Table 5), and SFA metal binding ability. SFA concentration does, however, have significant interactions with alum level and pH (BD, CD). The explanation for the apparent unimportance of SFA concentration is contained in these interaction effects.

The variable and interaction metal ion removal effects.  
Looking at the effects and F ratios in Table 6, the three

metal ions have different responses to the water treatment experiment, which is expected because of the dissimilarity of the metal ions. First, the different hydrolysis and SFA complexation properties of the three metal ions accounts for some of the variation. Copper hydrolyzes and complexes SFA to a much greater extent in the experimental conditions than cadmium or zinc (Table 2; Stumm & Morgan, 1970, p. 242). This is reflected in the greater range and average metal ion removal measured for copper compared to the other two ions, as already mentioned (Table 4). Only great effects can be detected for the small  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  losses. The cadmium effects range from -1 to 18% average change in metal ion removal. The zinc effects only range from -2 to 39% compared to the copper range of -23 to 38%.

Another consequence of the tiny response is to desensitize the ANOVA. With an adequate spread of measured removals from the high and low variable levels, the ANOVA easily detects significant changes, as in the case of the copper experiment. Where small changes occur, as in the cadmium experiment, the ANOVA is not so robust. In the cadmium experiment where random error is almost as great as the small percent metal removal changes, nearly all the variables and interactions appear significant at the 95% confidence level. For this reason only the effects which can be identified as significant with 99% certainty are considered in the ANOVA results.

One other dissimilarity of the three metal ions which

accounts for their different experimental behavior is their analytical differences. Specifically, zinc is more difficult to determine by AAS than copper and cadmium. As I indicated earlier, the metal ion experimental levels were chosen so that flame AAS could be used for analysis. The high zinc detection limits (ca. 100 ppb) were primarily responsible for the variable range chosen. More important, the poorer AAS signal to noise ratio for zinc compared to the other two metal ions limited the sensitivity of the zinc measurements. That is, it is more difficult to see small differences in zinc concentrations than in cadmium or copper levels by AAS. This means that if all the experimental effects were the same for the three metal ions, their analysis would affect the ANOVA results because the insensitive analyses would make distinctions more fuzzy: small effects would get lost in the analytical noise.

Table 6 has all the effects of the four experimental variables and their interactions. These effects are the difference between the average metal ion losses at the high and low level settings (Appendix 1). They measure the extent and the direction (sign) of the average metal ion loss change. In the following discussion, all of the ANOVA significant effects will be discussed beginning with the interaction effects that reveal potential metal ion removal mechanisms.

Effect of pH. Considering how important pH is to the metal binding ability of SFA and alum, and to the hydrolysis

of the analyte ions and alum, it is not surprising that this variable (C) and some interactions with this variable (BC and CD) significantly affect cadmium, copper and zinc removal in the water treatment experiment. The alum concentration-pH interaction (BC) reflects the hydrolysis and flocculation properties of aluminum that are pH dominated and involved in metal ion removal (Leentvaar, Buning & Koppers, 1978; Naylor & Dague, 1975; Linstedt, Houck & O'Connor, 1971; and others mentioned in the introduction to this chapter). In aluminum sulfate solutions more concentrated than 21 mg/L with pH between 5 and 7, most of the aluminum is in the insoluble  $\text{Al}(\text{OH})_3$  form (Hayden & Rubin, 1974). This hydrolyzed aluminum floc has a pH of zero point of charge of 5.0 (Stumm & Morgan, 1970), which means in the experimental pH conditions the floc has an anionic surface, not accounting for the surface activity of SFA, which is conducive to the electrostatic attraction of cations like  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and spectator cations ( $\text{K}^+$ ,  $\text{Na}^+$ ). In solution, the principal aluminum species above pH 7 is  $\text{Al}(\text{OH})_4^-$  (Rubin & Kovac, 1975). The overall effect of raising the pH from 5 to 7 is to increase aluminum hydrolysis and coagulation, which apparently corresponds to an increased copper, cadmium or zinc removal. In Table 6, factor BC is assigned a net increase of copper, cadmium and zinc removal by 22%, 10% and 29% respectively.

This interaction is shown in Figure 2 where the average percent copper removals for all experiments are plotted at

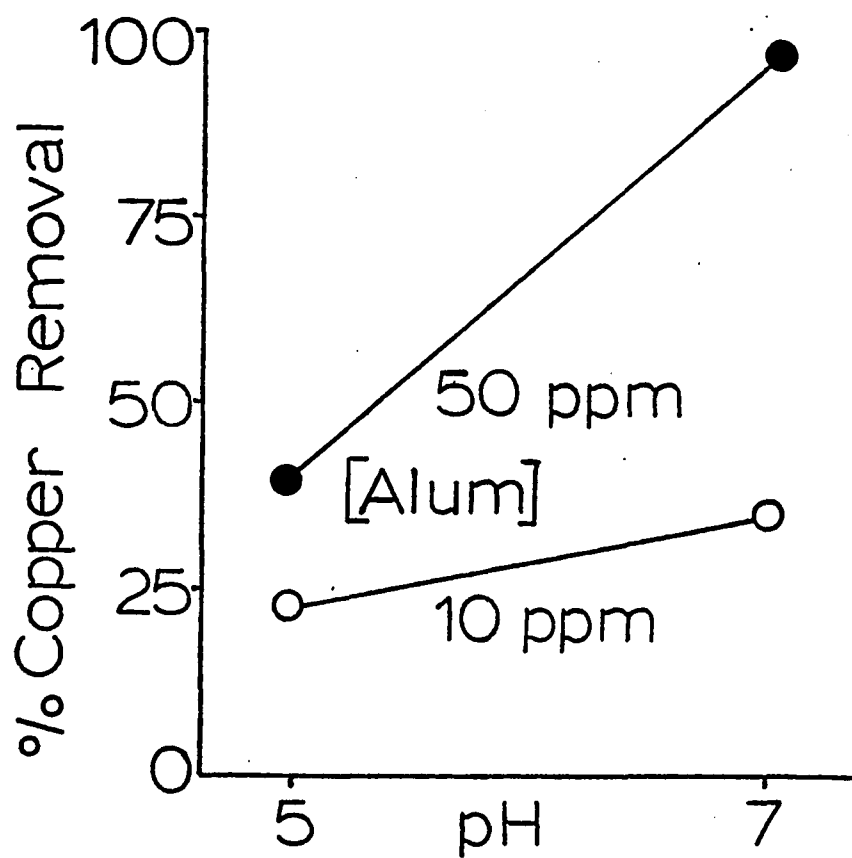


Figure 2. Interaction Effect of Alum Concentration and pH on  $\text{Cu}^{2+}$  Removal in the Water Treatment Experiment



pH 5 and 7 when a 10 mg/L alum dosage is used, and again when a 50 mg/L alum dosage is used. At the low alum level in this figure a small copper removal increase (11%) occurs when the pH is increased from 5 to 7, but at the high alum level the same pH change causes a much greater copper removal (55%). The greater effect at the higher alum levels is what the ANOVA calculations identify as a significant interaction effect. If there was no interaction, the same metal ion removal would occur from raising the pH at both alum dosages.

The ANOVA results in Table 6 reveal that the interaction of pH and SFA concentration is another significant effect in the removal of metal ions. This interaction, where -23%, 8% and 2% removal was effected for copper, cadmium and zinc respectively, is a function of the pH dependence of SFA-metal ion binding. The pH dependence of SFA-metal ion complex formation constants were reported by Bresnahan, Grant and Weber (1978) and Cheam and Gamble (1974). Approximately a four-fold increase in stability constants and a three-fold increase in the number of binding sites accompanies a pH change from 4 to 6 in the Cu-SFA system (Bresnahan et al., 1978). This pH influence is significant because the SFA complexation reaction dominates the speciation of  $\text{Cu}^{2+}$  and has some role in  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  speciation in the water treatment experiment. Measurements of SFA bound to  $\text{Cu}^{2+}$  versus free  $\text{Cu}^{2+}$  (where  $\text{Cu}^{2+}$  and SFA are both  $2 \times 10^{-4}$  M, pH is 6 and at 0.1 M ionic strength) reveal 96% of  $\text{Cu}^{2+}$  is SFA bound (Bres-

nahan, 1977). Under the same conditions, 53% of available  $\text{Cd}^{2+}$  is bound to SFA (Saar & Weber, 1979). The lesser role of SFA in  $\text{Cd}^{2+}$  speciation reflects the approximately  $10^2$  smaller conditional stability constant for  $\text{Cd}^{2+}$ -SFA. Zn-fulvic acid complexes are comparably weaker than the  $\text{Cu}^{2+}$  analogs (Mantoura, Dickson & Riley, 1978). The small CD effects for the cadmium and zinc experiments reflect their small SFA complexation ability (Table 6).

The negative SFA-pH interaction for the copper experiment is shown in Figure 3. In all the water treatment experiments where the SFA concentration was increased from 0 to 20 mg/L, at pH 5, an 18% increase in copper removal was effected, but at pH 7 a 27% decrease in copper removal is seen. Raising the pH to 7 where the metal ion is more strongly complexed with the fulvic acid and where the complex is more soluble, apparently has an antagonistic effect on the copper removal by alum coagulation. At pH 7 the fulvic acid succeeds in solubilizing metal ion that was removed from solution by alum coagulation when SFA is absent.

This interaction effect also serves to emphasize the benefit of the factorial experimental design. While there is a clear influence by the fulvic acid on metal ion removal, the ANOVA results (and the results of one variable at-a-time experimental procedures) indicate that overall, fulvic acid concentration changes had no significant net effect on metal ion removal (factor D in Table 6). In fact, this is a very important variable, as the interaction demon-

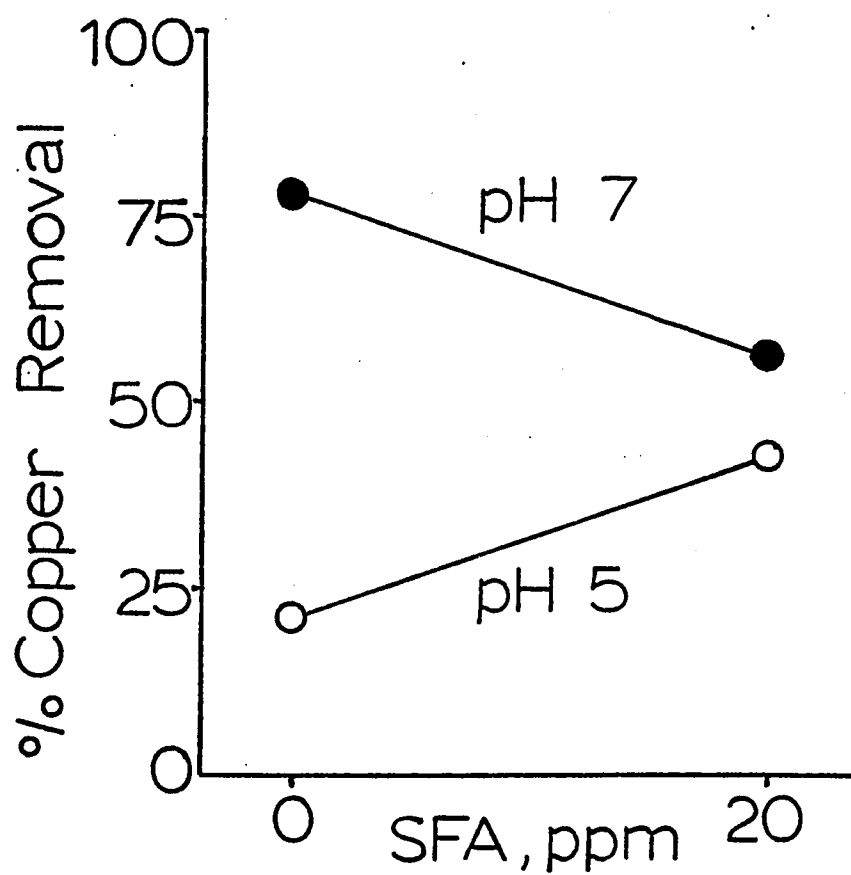


Figure 3. Interaction Effects of SFA Concentration and pH on  $\text{Cu}^{2+}$  Removal in the Water Treatment Experiment

strates. The positive interaction of alum level and SFA level (BD) opposes the CD effect, resulting in a small net SFA effect.

In contrast to the SFA concentration-pH interaction, changing pH has little interaction with changing metal ion level (AC), as seen in Table 6, where the insignificant effects are -8%, 4% and zero for copper, cadmium and zinc respectively. This interaction effect would be indicative of hydrolysis of  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  or  $\text{Cd}^{2+}$  to form insoluble  $\text{M}(\text{OH})_2$ , which is not expected under the experimental conditions (0.5 to 1 mg/L).  $\text{CuO}$  formation is expected from the copper hydrolysis tendency (Baes & Mesmer, 1976), and is observed in the water treatment background experiments, for the pH 7, 1 mg/L  $\text{Cu}^{2+}$  experiment with SFA absent. Other than that experiment, the predominant metal ion hydrolysis species for 1 mg/L metal ion concentration, no SFA present, and for pH 5 to 7 are  $\text{Cd}^{2+}$  (81 to 100%) and  $\text{Cd}(\text{OH})^+$  (0 to 19%), or  $\text{Zn}^{2+}$  (100%), or  $\text{Cu}^{2+}$  (60 to 100%),  $\text{Cu}(\text{OH})^+$  (0 to 30%), and  $\text{Cu}_2(\text{OH})_2^{2+}$  (0 to 10%) (Beneš & Kopečka, 1974; Patterson, Allen & Scala, 1977; Sylva, 1976).

#### Effects of Alum Concentration on Metal Ion Removal

Over all the experiments, changing the alum dosage caused a significant metal ion removal change of 65% for copper, 13% for cadmium and 30% for zinc (Table 6, factor B), and alum dosage change had significant interactions with pH as previously discussed (BC), and with SFA concentration (BD). While some metal ion removal is achieved by the alum

coagulation alone, the SFA-alum dose interaction indicates that some metal ion removal proceeds in conjunction with SFA removal. Alum coagulation is an established, efficient procedure for removing organic matter (color) from solution (Hall & Packham, 1965; Gauntlett & Packham, 1973; Leentvaar, Buning & Koppers, 1977; Narkis & Rebhum, 1975 and 1977). Indeed, the water treatment experiment results show very good color removal by 30 and 50 mg/L alum doses on average, although the 10 mg/L dose caused virtually no color removal (Table 5). The metal ion removals measured in the water treatment experiments can be linked to SFA removal considering SFA-metal binding ability and the increased metal removal attributed to the BD effect for all three metal ions (Table 6).

In the case of copper removal, the SFA-alum dose interaction (factor BD in Table 6) is shown in Figure 4. In this figure a comparison of metal ion losses with increasing SFA level shows a dependence of metal ion removal on alum dosage. At the 10 mg/L alum dose, raising the fulvic acid concentration causes a 23% decrease in copper removal, but copper removal increases 13% over the same fulvic acid concentration range at the 50 mg/L alum level. Alum concentration and fulvic acid concentration clearly interact to cause changes in metal ion removal efficiencies.

The effects shown in Figure 4 are the consequence of the metal binding ability of fulvic acid and the color removing ability of alum coagulation. When the alum dosage is in-

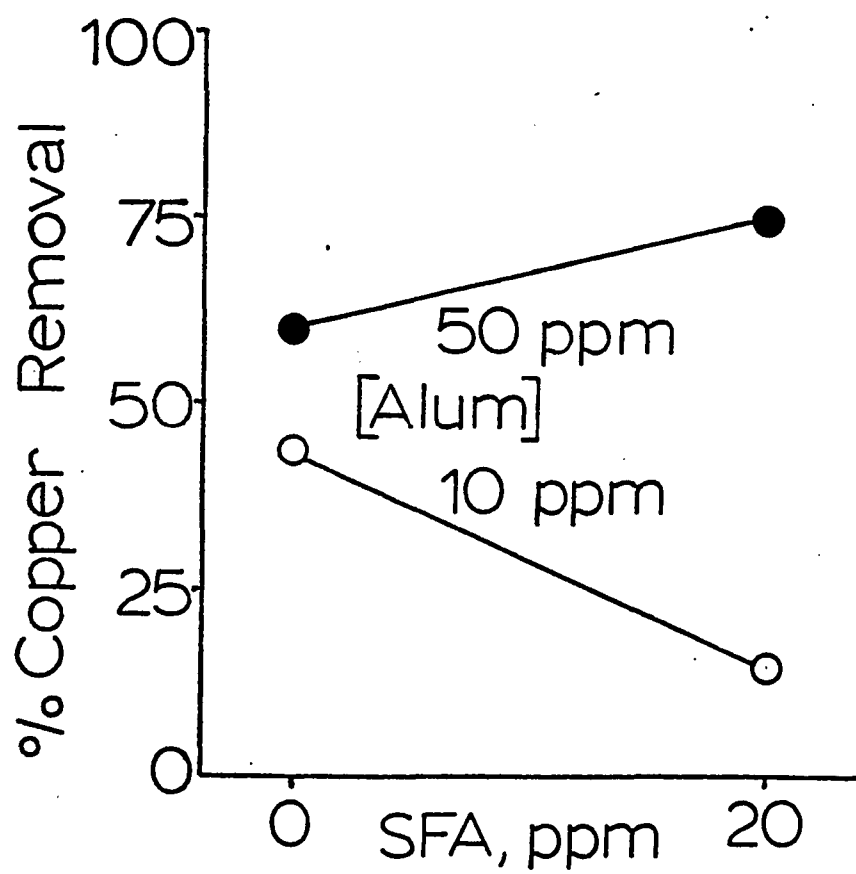


Figure 4. Interaction Effect of Alum and SFA Concentrations on  $\text{Cu}^{2+}$  Removal in the Water Treatment Experiment

sufficient to totally remove the humic matter, as it was at the 10 mg/L dosage, the metal complexing fulvic acid desorbs metal ions from the hydrolyzed aluminum floc to form soluble metal complexes. Hence the lowered copper removal in Figure 4 for the low alum level. On the other hand, when the coagulation process effectively removes all the fulvic acid, as is the case for the 50 mg/L alum concentration experiments, the metal ions removed by alum coagulation are not resolubilized. In fact, the alum adsorbed humic matter enhances metal ion removal, either because the metal complex is adsorbed and removed or because the adsorbed fulvic acid scavenges metal ions from solution by complexation as it is filtered out of solution with the alum floc. These processes are enhanced by raising the pH because SFA complexation ability increases and alum hydrolysis increases with pH, thus the largest metal ion losses generally occur for high alum, high SFA and high pH level experiments (Table 3, experiments 1 and 9).

#### Main Variable Effects

Once the interactions have been examined, the effects of the four main variables can be understood. The net effects of the significant water treatment experiment variables and their interactions on metal ion removal is given by the 99% confidence level F ratios of the single factors A (metal ion level), B (alum concentration), C (pH), D (SFA level) in Table 6. The net copper removal effect of raising each of these variables from their low to their high setting

is shown in Figure 5. As the ANOVA results indicate, copper ion levels from .1 to 0.5 mg/L are removed equally well, i.e., changing variable A in the water treatment experiments had no discernable effect on metal losses due to the coagulation process. As was already discussed, changing the fulvic acid level from 0 to 20 mg/L had an important effect on metal ion removal, but the net effect over all the experiments in the factorial design was minimal due to opposed interaction effects. On the basis of the single variable effects alone, the actual contribution of fulvic acid to the metal ion removal would be discounted, which demonstrates both the value of elucidating the interaction effects with ANOVA and factorial design and the potential error of accepting the main variable effects without considering significant interactions. Finally, Figure 5 shows the 33% and 38% net increase in copper removal caused by increasing the pH and alum levels in all the water treatment experiments. These large increases reflect the large effects of factors B and C in Table 6.

In summary, three observations should be emphasized from the metal ion and fulvic acid loss results. First, alum coagulation causes some  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  removal from solution (Figure 2) in agreement with other studies. Second, SFA alone is not responsible for any metal ion losses in the coagulation processes, as was shown in the background metal loss study. Third, fulvic acid significantly influences  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  removal during the alum coagulation process in conjunction with solution pH and alum level (Figures



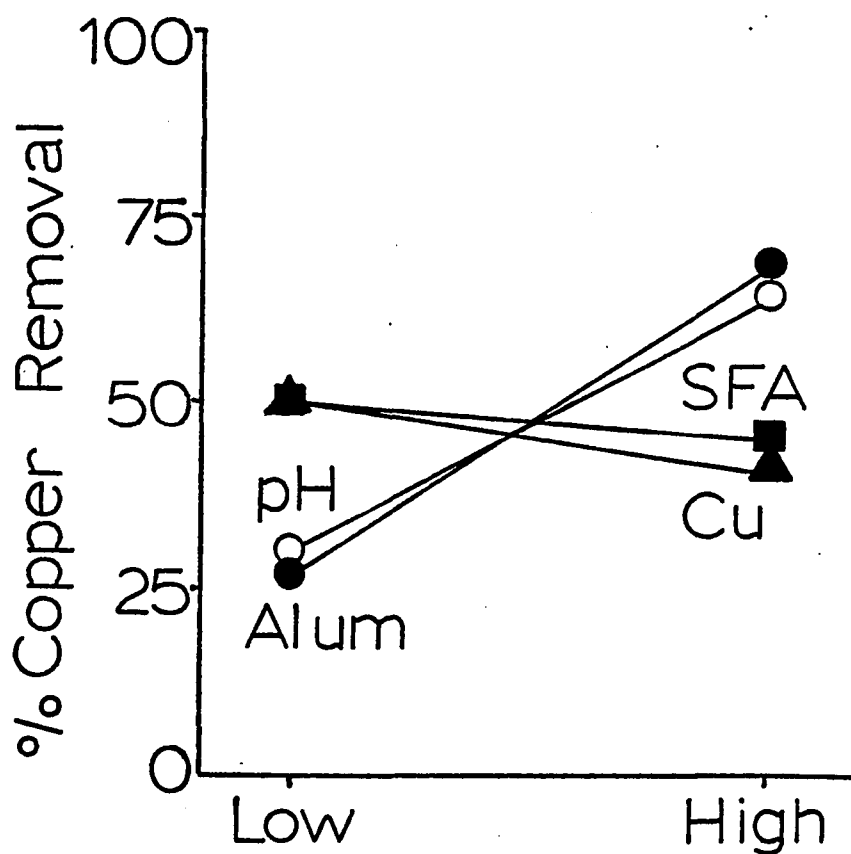


Figure 5. Overall Effects of Alum Dosage, pH Level, Copper Concentration, and SFA Concentration on  $\text{Cu}^{2+}$  Removal in the Water Treatment Experiment

3 and 4). The SFA effect is a mixed blessing, however. When all the SFA is removed from solution by alum coagulation, SFA promotes cadmium, copper and zinc ion removal. When SFA is left in solution, it acts to keep these metal ions in solution. This last observation is important because humic compounds are commonly found in drinking water supplies at the levels studied in the water treatment experiment (Reuter & Perdue, 1977).

### Conclusions

A likely mode of metal ion removal during alum precipitation in the presence of fulvic acid can be deduced from the water treatment experiment results. Three possible mechanisms are precipitation of a metal moiety independent of alum flocculation, coprecipitation of an insoluble metal species with the alum floc, and association of a soluble metal species to the alum floc or to SFA adsorbed to the alum floc. The water treatment ANOVA results and the background metal ion loss experiments provide evidence to suggest the last mechanism is most important.

The predominant dissolved metal species expected in these experiments are metal hydroxo complexes ( $M(OH)^+$ ), simple hydrated metal ions ( $M^{2+}$ ) and soluble metal fulvate complexes (MSFA) (Stumm & Morgan, 1970). A minor exception is the presence of some insoluble  $CuO$ , as previously discussed. Indeed, no precipitated metal ion species is observed in the background metal ion loss experiments with one

exception as noted. As a result, this mode of metal ion removal during the alum coagulation treatment is discounted. For these same reasons, no alum coprecipitation of insoluble metal species is expected.

Small metal ion losses, observed in the background filtration experiments, which are due to manipulation of the test solutions, account for some of the water treatment experiment results, but the most important mode of metal ion removal is the adsorption of  $M^{2+}$ ,  $M(OH)^+$  and/or MSFA on the precipitated alum floc.

Considering the significant interaction of alum dosage and SFA level (factor BD, Table 6), and interactions of these variables with pH, and the metal binding ability of SFA, the principal metal removal mechanism involves the adsorption of MSFA complexes on the alum floc. In this mechanism, the coagulating aluminum hydroxide effectively adsorbs dissolved SFA ions, which in turn complex dissolved metal ions. Alternatively, the metal complex associates with the alum floc. The copper, cadmium and zinc ions are then separated from solution along with the alum floc during filtration. Note as evidence that metal ions are much less effectively removed from solution in the absence of SFA in most cases (Table 3). Therefore, the metal scavenging effect of adsorbed SFA is quite significant in metal ion removal. Also noteworthy is the ability of SFA to re-dissolve metal ions that ordinarily would be removed by the alum coagulation process when SFA is left in solution. An important conclusion:

in terms of removing metal ions from drinking water, it would seem that complete color removal is required to insure that metals are not transported by humic materials through the water treatment processes to the public.

There are other consequences of metal ion removal by adsorbed humic matter in this treatment process. The efficiency of this removal mechanism is dependent on the extent of  $M^{2+}$ -SFA binding. Thus,  $Cu^{2+}$ ,  $Pb^{2+}$  and  $Hg^{2+}$ , which are strongly bound metal ions, are more effectively removed than ions like  $Zn^{2+}$  and  $Cd^{2+}$ . Indeed, in the water treatment experiment,  $Cd^{2+}$  is not effectively removed under any conditions. Considering the toxicity of this ion, the ineffectiveness of  $Cd^{2+}$  removal in typical water treatment conditions is a matter of concern. Finally, while SFA binding increases with pH between pH 5 and 7, metal ion removal at pH greater than 7 would probably decrease as the continued hydrolysis of aluminum produces soluble polynuclear aluminum cations (e.g.,  $Al_2(OH)_2^{+4}$ , Rubin & Kovac, 1975), thereby decreasing the concentration of insoluble alum species.

## CHAPTER 3

### TRACE METAL ION FILTRATION LOSSES AT pH 5 AND 7

#### Introduction

An important experimental consideration discussed in the water treatment experiment is the ability to filter trace metal solutions at pH 5 to 7 with insignificant metal ion losses to container walls and the various materials each solution encounters during the filtration process. Obviously these metal ion losses, if they are large, invalidate the results of experiments designed to measure losses due to the action of alum coagulation. Metal ion losses during sample filtration are also a problem in the complexing capacity studies described in the next chapter, where natural water samples are filtered prior to an analysis of their metal ion equilibria.

Microfiltering (ca. 0.4  $\mu\text{m}$  pore size) trace metal ion solutions at high pH without changing their metal ion levels is experimentally difficult to meet with conventional filtration apparatus and filtration procedures because metal ion contamination and sorption losses can occur from contact with all the materials encountered during filtration, dilution, storage and other pre-analysis sample manipulations. Maintaining sample integrity during microfiltration is particularly difficult in metal ion speciation

studies with media such as lake water (Chau & Lum-Shue-Chan, 1974), wastewater (Kunkel & Manahan, 1973), estuarine water (Hart & Davies, 1977), seawater (Batley & Florence, 1976b), and river water (Ramamoorthy & Kushner, 1975; Stella & Ganzerli-Valentini, 1979), where the sample cannot be acidified prior to filtration. While the effect of filtration on these samples is not reported by the authors, others have acknowledged the problems of filtering environmental water samples (Beneš & Steinnes, 1974; Batley & Gardiner, 1977; Salim & Cooksey, 1979). No procedure to avoid these problems has been demonstrated.

Some investigators have considered microfiltration metal contaminations and losses in some detail. Nürnberg et al. (1976) reported 70%  $\text{Cd}^{2+}$  and 88%  $\text{Pb}^{2+}$  losses from approximately 0.1 ppb metal ion solutions when untreated filter membranes were used. Losses of  $\text{Cd}^{2+}$  (Beneš & Kopicčka, 1976; King, Rodriguez & Wai, 1974; Gardiner, 1974),  $\text{Pb}^{2+}$  (Issaq & Zielinski, 1974), and other elements (Struempler, 1973; Robertson, 1968) are greater for glass than polyethylene or polypropylene, and increase at higher pH. A study by Beneš et al. (1975) also demonstrated that sorption losses vary from ion to ion. To minimize losses or contaminations in trace metal analyses, the use of acid washed vessels, non-sorbing materials, and using the fewest possible sample manipulations are generally recommended procedures (Florence & Batley, 1977; Zief & Mitchell, 1976; Tögl, 1972).

In this chapter a microfiltration filtration apparatus

was developed and evaluated. In some of my preliminary filtration experiments,  $\text{Cd}^{2+}$  losses to a variety of filter media were found (Truitt & Weber, 1979a). Losses from 125 ng/mL pH 8 solution were 29% with Whatman #2 paper filters, 35% with Millipore cellulose acetate filters, and 16% with Nucleopore polycarbonate filters. Medium (10-15  $\mu\text{m}$ ) and fine (4-5.5  $\mu\text{m}$ ) porosity 40-mm sintered glass filters caused 60% losses with 99 ng/mL pH 8  $\text{Cd}^{2+}$  solutions. These preliminary experiments serve to demonstrate the extent of metal ion losses possible and the variation of losses with different filter materials that can be encountered.

So that the water treatment experiment could be run and natural water samples prepared for analysis, a filter apparatus was built to minimize metal ion losses during microfiltration. The apparatus and the performance of different filter supports and membranes were evaluated in a series of factorial designed experiments. As well as quantifying the effect of filtering high pH trace metal ion solutions with this apparatus, the filtration experiment will also serve to emphasize that metal ion concentration changes can occur when solutions with pH and metal ion levels similar to many natural water systems are filtered.

### Experimental

#### Materials and Equipment

Standard metal ion solutions were prepared by diluting new Fisher AAS 1000 ppm standard solutions. The solid soil

fulvic acid (SFA) was isolated as previously detailed (Weber & Wilson, 1975). Deionized, distilled water was used throughout. All solutions were prepared in polypropylene volumetric flasks (Nalgene Corp., Rochester, N.Y.). All containers were soaked in 1 M  $\text{HNO}_3$  for 12 hours, then rinsed with distilled water prior to use. An Orion 701 digital meter with a 5 mm o.d. Vanlab combination electrode (#34106-079) was used to measure pH. Metal ion determinations were made by flame AAS using a Techtron AA5 spectrometer and a Heath model EU-205-11 strip chart recorder. SFA was detected at 260 nm with a Cary 14 spectrophotometer using 2 cm quartz cells.

Filtrations were performed using a glass filter assembly or a polycarbonate-Tygon tubing assembly. The plastic assembly (Figure 6) consists of a Nuclepore Filter Funnel Assembly (Nuclepore Corp., Pleasanton, Ca.) and a 25 cm tall wide-mouth bottle that is capped with a two-hole #14 rubber stopper. The filter funnel passes through one hole of the stopper and empties into a 200 mL polypropylene collection bottle through a short length of Tygon tubing. The other stopper hole contains a glass tube through which the wide-mouth bottle is evacuated with an aspirator. The bottom of the rubber stopper was wrapped in Parafilm to prevent rubber debris from falling into the collection bottle. The glass assembly consists of a Millipore Pyrex Filter Holder (Millipore Filter Corp., Bedford, Ma.) emptying into a 125 mL Pyrex filter flask.

Metal ion determinations were made by DPASV/HMDE using



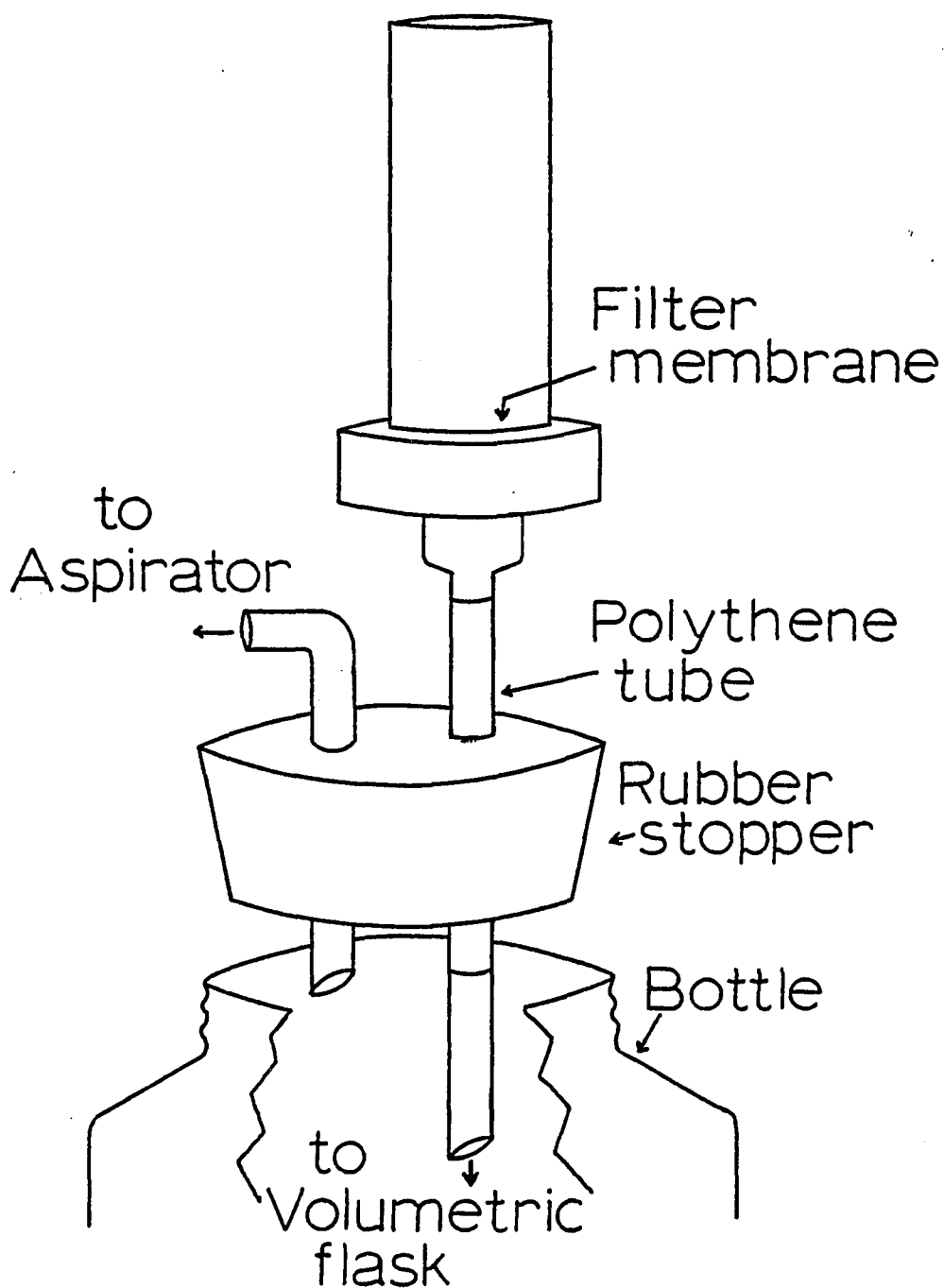


Figure 6. Drawing of the All-Plastic Microfiltration Apparatus

a PAR 174A Polarographic Analyzer, a PAR 315 Electroanalysis Controller, and a PAR 9323 Hanging Mercury Drop Electrode (Princeton Applied Research, Princeton, N.J.).

#### Comparison of Filter Assemblies

$\text{Cu}^{2+}$  filtration losses were measured as a function of three variables: pH, the filter membrane used, and the filter support used. To assess the relative effect of these variables and their interactions, the  $\text{Cu}^{2+}$  filtration losses were determined using a  $2^3$  factorial experimental design (Table 7). Metal ion losses with Nuclepore 47 mm polycarbonate 0.4  $\mu\text{m}$  porosity membranes (n) and Gelman Metrical 47 mm cellulose tri-acetate 0.45  $\mu\text{m}$  porosity membranes (m), the polycarbonate filter support just described (N) and a glass filter support (M), and pH 5 and 7 solutions were measured. Metal ion determinations were made within 5 hr of the filtration. The experiments were performed in duplicate and in random order and the experimental results were evaluated by analysis of variance (ANOVA).

Stock 25 mg/mL  $\text{Cu}^{2+}$  solutions ( $\mu=0.01$ ) were made in acetate buffer (pH 5) and in phosphate buffer (pH 7), and  $\text{Cu}^{2+}$  losses were measured using the following procedure. With a pre-rinsed pipet, a 50 mL aliquot of the appropriate stock solution was delivered to the filter apparatus. After filtration the filtrate was collected in 200 mL polypropylene bottles and acidified with 50  $\mu\text{L}$  reagent  $\text{HNO}_3$ . The filter assembly was prepared for the next experiment by discarding the used membrane, rinsing the assembly with 500 mL 1  $\text{M}$   $\text{HNO}_3$ ,

Table 7. Percent Copper Loss From Filtering 25  $\mu\text{g/L}$  Copper Solutions as a Function of pH and Filter Apparatus

<u>Filter<sup>a</sup> Support</u>	<u>Filter<sup>a</sup> Membrane</u>	<u>pH</u>	<u>Average % Cu Loss</u>
M	m	5	29
M	m	7	25
M	n	5	60
M	n	7	79
N	m	5	13
N	m	7	5
N	n	5	2
N	n	7	0

<sup>a</sup>N and n = polycarbonate; M = glass; m = cellulose acetate

and then rinsing with 500 mL water. A pair of unfiltered control solutions were included in the experimental design by delivering 50 mL aliquots from each stock solution directly to the collection bottles. The control solutions provided a measurement of the actual metal ion concentration of the stock buffer solutions during the filtration experiment.

Using the same procedure, 50 mL metal-free pH 5 buffer and pH 7 buffer aliquots were filtered with the polycarbonate apparatus. Both polycarbonate and cellulose acetate membranes were used to determine the extent of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  contamination from the membranes.

### Results and Discussion

Metal ion contaminations and losses were distinguished by two sets of filtration experiments. In the first set acetate buffer or phosphate buffer was filtered using cellulose acetate or polycarbonate membranes on the polycarbonate support so that the presence of leachable metal ions could be measured. Buffers were used so that the pH could be controlled during the filtration. Slight contaminations of  $\text{Cu}^{2+}$  (2 ng/mL for pH 5 buffer and 2-7 ng/mL for pH 7 buffer) and  $\text{Pb}^{2+}$  (0 ng/mL for pH 5 buffer and 1 ng/mL for pH 7 buffer) were detected with both membranes. There was great variation in contamination over five replicate filtrations (relative standard deviations up to 100%), but clearly  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  leach from both membranes at pH 5 and 7. For confirmational evidence, Wallace et al. (1972) reported that

acid washing leaches copper and lead from Nuclepore membranes. They also reported that acid washing reduces residual copper and lead levels 68% compared to water washing.

In the second series of experiments, percentage  $\text{Cu}^{2+}$  losses were determined as a function of filter support, filter membrane, and pH. The percentage losses in Table 7 are relative to the original  $\text{Cu}^{2+}$  concentrations. Filtration losses ranged from 79 to 0% ( $\pm 12\%$ ). Note that the greatest losses occurred using the glass support (M). On the other hand, except for filtering with a cellulose acetate membrane (m) at pH 5 where a 12.5% loss was measured, losses with the polycarbonate support (N) were insignificant. Clearly, the most important factor determining  $\text{Cu}^{2+}$  losses is the large difference between the glass and polycarbonate supports.

The ANOVA results in Table 8 reveal other important aspects of the filtration experiment. Although the effect of the different supports was by far the greatest, the choice of membrane and the support-membrane interaction are also significant effects at the 95% confidence interval. The importance of membrane choice reflects the larger average  $\text{Cu}^{2+}$  losses with polycarbonate membranes (35%) than with cellulose acetate membranes (18%). The membrane effect (Table 8) is negative because the polycarbonate membranes were arbitrarily designated as the low (-) membrane setting in the experimental design. The significance of the support-membrane interaction arises from the higher  $\text{Cu}^{2+}$  losses that occurred with glass-polycarbonate (Mn) and polycarbo-

Table 8. ANOVA Results of the  $2^3$  Factorial Filtration Experiment

<u>Variable</u>	<u>Effects</u>	<u>F Ratio</u>
Support	43.25	57 <sup>a</sup>
Membrane	-17.25	9 <sup>a</sup>
pH	-1.25	0
<u>Interactions</u>		
Support-Membrane	-25.25	19 <sup>a</sup>
Support-pH	-6.25	1.2
pH-Membrane	7.25	1.7
Support-Membrane-pH	4.25	0.6

<sup>a</sup>F ratios significant at the 95% confidence level  
( $F_{95}(1,8)=5.3$ ).

nate-cellulose acetate (Nm) apparatus compared to the glass-cellulose acetate and all polycarbonate combinations. The average  $\text{Cu}^{2+}$  loss of Mn and Nm combinations was 39% compared to 14% for Mm and Nn apparatus. All other variable interactions had little effect on  $\text{Cu}^{2+}$  losses. The insignificance of varying pH from 5 to 7 indicates that no effect at the 25  $\mu\text{g/L}$  level could be seen due to any copper hydrolysis and colloid formation.

### Conclusion

Filtering trace  $\text{Cu}^{2+}$  solutions at pH 5 and 7 can result in large changes in metal ion concentration. Glass filter supports should be avoided for natural water filtration because large metal ion adsorption losses occur. An all plastic support such as the one I constructed for this study causes much smaller metal ion losses. Both cellulose acetate (Gelman) and polycarbonate (Nuclepore) 0.4  $\mu\text{m}$  filter membranes leach  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  into phosphate and acetate buffer solutions that approximate natural water pH. The leached  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  levels are in some cases as high as natural levels in freshwater samples, indicating a potential serious contamination problem. In summary, typical microfiltration techniques at environmental sample pH levels can significantly alter trace metal ion concentrations, and it is a mistake to ignore the effect of these manipulations on metal ion concentrations. The contamination and metal ion loss problems can be minimized by acid rinsing the filter membranes (Wallace

et al., 1972) or conditioning them with sample solution (Nürnberg et al., 1976), and by using an acid-washed, all-plastic filtration system.



## CHAPTER 4

### THE COMPLEXING CAPACITY OF FULVIC ACID AND NATURAL WATER SAMPLES

#### Introduction

The water treatment experiment presented in Chapter 2 demonstrated the ability of humic matter to transport metal ions through drinking water treatment processes, where they become potential public health hazards. As a result, the chemical fate of metal ions that are introduced to natural water systems either by natural mechanisms, such as weathering of minerals or man-made activities, is worthy of investigation. The development and evaluation of an analytical methodology to determine the chemical equilibrium forms of metal ions in natural water systems is the aim of the study described in this chapter.

#### Definition of Natural Water Metal Complexing Capacity

Efforts are made to understand the state of metal species in the aquatic environment because of the toxicity of some species to aquatic life and to man (Wood, 1974; Fowler, 1974). The free (uncomplexed) transition metal ions are the object of such studies since they are often the most toxic metal species. "Complexing capacity" is a characteristic of natural water systems that has been adopted by some researchers as a measure of the metal ion

binding ability of the water, much the same way alkalinity measures proton binding ability. Complexing capacity measures regulation of free metal ion levels by naturally occurring ligands.

The term "complexing capacity" is inconsistently defined in the literature. For example, a few researchers include sorption to suspended material in the measurement of free metal ion regulations mechanisms, recognizing that sorption to clays, colloidal material and sediment is the usual fate of many metal ions (Tessier et al., 1979). However, while inclusion of adsorption phenomena is a more realistic model of natural water, the resulting samples are experimentally complex. Also, metal ions associated with suspended material are usually removed during drinking water treatment processes. For these reasons, most researchers are careful to first remove suspended material by microfiltration (ca. 0.4  $\mu\text{m}$  porosity) to isolate dissolved species and greatly simplify the experimental system. In any case, "complexing capacity" is measured by some kind of titration of the water sample with the metal ion of interest. The titration endpoint is detected by the sudden increase in uncomplexed metal ion when the binding ability of the water sample is exhausted.

The inconsistency and resulting limited usefulness of the term "complexing capacity" arises from its pragmatic operational definitions. The study of trace metal ion

species equilibria in complicated, undefined natural water media is so difficult, "complexing capacity" is often defined in such a way as to simplify its measurement. As a result, these measurements are a function of methodology as much as a characteristic of the water sample. These operational definitions make comparisons of different complexing capacity studies difficult. Definitions based upon the analytical technique employed are often incomparable because sample manipulation is required (e.g., addition of electrolyte, changing pH), and metal ion specificity is different among the methods employed.

Finally, none of the adopted definitions distinguishes between the amount of the complexing capacity that has already been expended by naturally occurring metal ions and the unexpended fraction of the capacity which is available for further complexation. This last unused portion of the capacity is the most interesting and is in fact what is commonly measured and called the complexing capacity. For consistency and accuracy, a theoretical definition of complexing capacity will be made for use in this study.

In its use in this dissertation, the term complexing capacity (CC) is defined as the amount of metal ion binding material present in solution. It refers to a specific metal ion or group of metal ions and is expressed as mg/L or micromolarity of bound species. The complexing capacity has two components. The portion of the capacity already used by naturally occurring metal ions is called the natu-

ral complexation level (NCL). The remaining fraction still available for complexation is called the potential complexation level (PCL).

Experimentally, the difference between total metal ion concentration and unbound, free metal ion concentration is the NCL of the system. In a titration where metal ions are added to the water sample, the point where no more metal ion is bound marks the complexing capacity of the sample. PCL is the CC less the NCL.

I emphasize that a complexing capacity measurement refers to a particular metal ion or group of ions, (e.g., the copper(II) complexing capacity or the alkaline earth complexing capacity), since metal ions can be bound to varying extents. Four typical situations are diagrammed in Figure 7. In the first case ( $M_1$ ), the metal ion is in great natural abundance, but has a weak affinity for the complexing agents in the water system. As a result, NCL is a large fraction of the capacity and the complexing capacity is small. The alkali metals, alkaline earth metals and  $Zn^{2+}$  are examples of this first case. The second case ( $M_2$ ) is where the metal ion has a strong affinity for the natural ligands (large CC) and the natural metal ion level has used most of the capacity (large NCL).  $Fe^{3+}$  and  $Al^{3+}$  might be examples of the second case. The third case ( $M_3$ ) includes metal ions with low natural occurrence and weak affinity for complexation, e.g.,  $Cd^{2+}$  and  $Tl^+$ . The fourth category ( $M_4$ ) has the strongly complexing, low natural abun-

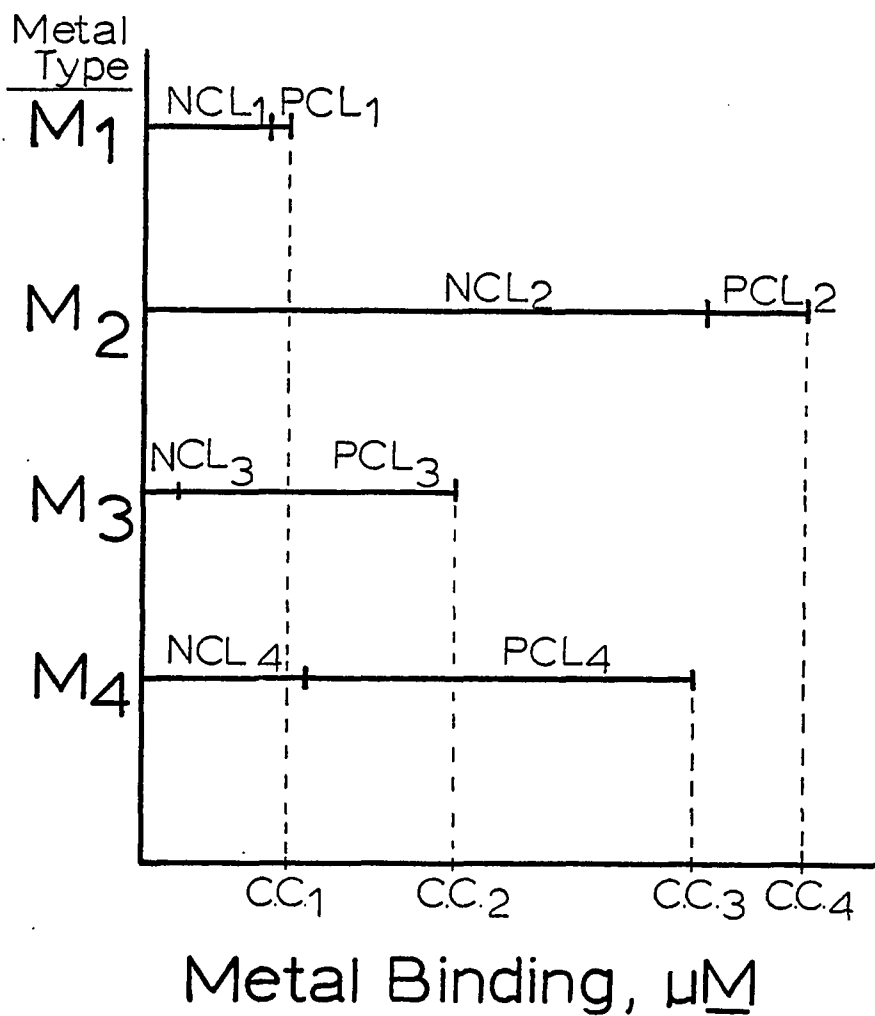


Figure 7. Metal Ion Classification Based Upon Binding Properties in Natural Freshwater Samples. NCL is Natural Complexing Level, PCL is Potential Complexing Level, and CC is Complexing Capacity.

dance metal ions like  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$ . This group contains many of the toxicologically important heavy metals and those ions that have received the most attention in complexing capacity studies. In Figure 7 the relative complexing capacities of the four cases are  $M_2 > M_4 > M_3 > M_1$ . The relative NCL's are  $M_2 > M_4 > M_1 > M_3$ , while the PCL's are  $M_4 > M_3 > M_2 > M_1$ .

### Literature Review

The analytical difficulty inherent with complexing capacity measurements is to distinguish the free metal species from the complexed species, including labile complexed metal ions, and at the same time have minimal effect on the chemical equilibrium under study. In the past, many techniques have been employed to make these exacting measurements. These complexing capacity determinations can be generally categorized as biological and chemical methods. Biological methods are much less harsh on the natural water equilibria than chemical methods, but offer a smaller range of metal ions that may be studied. Chemical methods offer precision, convenience, sensitivity and a greater number of metal ions that may be studied. A literature review of previous complexing capacity studies follows, where methodology will be emphasized and criticized.

Biological methods. Lewis et al. (1972) and Davey et al. (1973) measured natural water metal ion binding capacity semi-quantitatively by biological means. The method origi-

nates from observations of some organisms responding to free metal ions in their environment with inhibited growth or mortality in their early development stages. In these cases, the addition of metal sequestering agents such as clays, humic material, or EDTA cause organism survival or growth, thereby demonstrating the relative toxicity of the free ion compared to the sequestered species. Stolzberg and Rosin (1977) speculate that some phytoplankton react by releasing "extracellular metal binding organic matter" when subjected to metal ion stress. The organisms' motivation for sequestering environmental metal ions can be to either remove a toxic metal species or to collect a nutritive metal ion during a deficiency. Stolzberg (1977) suggested a polarographic measurement of these ligands.

Lewis et al. (1972) measured the  $\text{Cu}^{2+}$  binding capacity of humic material, sewage effluent, clays, EDTA and phytoplankton semi-quantitatively by first adding a sufficient quantity of  $\text{Cu}^{2+}$  to decrease the survival of Euchaeta japonica, then adding the test agent while monitoring the organism survival by bioassay. Davey et al. (1973) noted the similarity of this bioassay response of Thalassiosira pseudonana to a  $\text{Cu}^{2+}$  selective electrode in an EDTA titration of 0-50 ppb  $\text{Cu}^{2+}$  seawater. The organism growth is inhibited by as little as 1 ppb  $\text{Cu}^{2+}$ . However, due to their semi-quantitative nature and their specificity to some toxic metal species, the above bioassay methods have not been generally adopted.

On the other hand, a recent biological binding capacity determination by Gächter et al. (1978) is quantitative in nature. They monitored the phytoplankton photosynthesis rate, measured as  $^{14}\text{CO}_2$  evolved, after introducing  $^{14}\text{C}$  labelled  $\text{NaHCO}_3$  to the phytoplankton medium. The diagnostic metal ion binding behavior of the phytoplankton comes from the specific toxicity of  $\text{Cu}^{2+}$  and the ability of the organism to discriminate against all inorganically and organically complexed forms of the metal ion. Gächter et al. (1978) report a natural phytoplankton photosynthetic inhibitory effect from as little as  $10^{-11} \text{ M Cu}^{2+}$ . This specificity was demonstrated, as in previous bioassay studies, by comparing titrations of the phytoplankton medium with  $\text{Cu}^{2+}$  in the presence and absence of EDTA. Isotoxic copper(II) concentrations of the two titration curves were different by the EDTA equivalence of the second solution, indicating the phytoplankton discrimination of bound and free copper ion.

Solubilization method. In some chemical complexing capacity measurements, the fragility of natural water equilibria has been underestimated. One such study by Kunkel and Manahan (1973) sought to distinguish organically complexed  $\text{Cu}^{2+}$  from all other species based upon the insolubility of inorganic  $\text{Cu}^{2+}$  in the pH range 10 to 11. They proposed that in a copper ion titration experiment any  $\text{Cu}^{2+}$  concentration above  $2.4 \times 10^{-7} \text{ M}$  at pH 10-11 is due to solubilization of the metal ion by the natural complexing ligands. The excess  $\text{Cu}^{2+}$  concentration was therefore the



complexing capacity of the water sample. In a critical evaluation of this method, Campbell et al. (1977) found some difficulties with the  $\text{Cu}^{2+}$  solubilization method compared to ASV titration and zinc colorimetric methods. First, the calculated complexing capacity of monomeric model ligands like aspartic acid, salicylic acid, glycine and lysine are below the detection limit ( $3 \times 10^{-7}$  M) of the solubilization technique. Next, the calculated binding capacity of these model monomeric ligands is substantially different at pH 10 than at natural pH levels of 5-8. The solubilization method ignores the equilibrium disturbance of  $10^3$  to  $10^4$  change in proton activity during the analysis. Finally, ultrafiltration and nephelometry reveal that the "dissolved" copper(II) fraction of a humic acid complexing capacity measurement is in fact mostly colloidal. While some  $\text{Cu}^{2+}$  might be lost from solution by association or coprecipitation with the colloidal and particulate phases, the consequence of describing colloidal Cu(II) as solubilized complex results in erroneously high complexing capacity measurements. A difficulty of the Kunkel and Manahan procedure not mentioned by Campbell et al. (1977) is the unknown damage to the sample equilibrium and ligand structures from heating the sample to  $100^\circ\text{C}$  for one hour prior to separating dissolved from soluble fractions.

Cobalt complexation method. Hanck and Dillard (1977) and Dillard (1976) proposed another technique which ignores the potential sensitivity of natural equilibria. They take

advantage of the disparity of complex dissociation kinetics between Co(II) and Co(III). In this procedure, Co(II) is added to the sample in ten-fold excess of the ligand binding capacity. A small amount of  $H_2O_2$  is added to oxidize the metal ion to the inert Co(III) form, then enzyme catalase was added at 36°C to reduce the excess peroxide. The reaction hypothesized is the rapid and complete complexation of the Co(III) by the ligands in the water sample. The uncomplexed Co(III) is spontaneously reduced by water back to Co(II). The relative concentrations of the two species is calculated from polarographic measurement of the Co(II) concentration as  $Co(ethylenediamine)^{2+}$ .

There are three problems with the Co(II) complexation method. The first problem concerns the parameter being measured and called the "complexing capacity." The exhaustive complexation of natural water ligands by Co(III) would measure the total complexing capacity of the system. The binding capacity for any individual metal ion is not obtained from this technique however, and there is no means of measuring NCL or PCL for any individual metal ion. The second problem with this technique arises from the large complex formation constants assumed for Co(III) complexation. The technique requires Co(III) complex formation to be more favored over all other metal ions present to insure complete complexation. While this assumption rests on the huge Co(III) EDTA and Co(III) PDTA formation constants ( $1.5 \times 10^{38}$  and  $2.1 \times 10^{38}$  respectively (Dillard, 1976)), Hanck and

Dillard (1977) admit that further work in this area is needed. Assuming, however, that Co(III) does successfully compete with bound metals, there is still no guarantee that the ligands complexing Co(III) would necessarily complex other metal ions. In other words, what this technique really measures is specifically the Co(III) complexing capacity of a water sample. Finally, the proposed additions of  $H_2O_2$ , however small, could oxidize the metal binding organic matter and thereby alter the complexing capacity, or could oxidize naturally occurring metal ions, thereby altering the metal complex equilibria under study. The assumed specific oxidation properties of  $H_2O_2$  is not proven. The proposed addition of enzyme catalase also has an unknown influence on the metal speciation by complexation, although only  $10^{-10}$  moles of the enzyme were used.

Chromatographic methods. Van den Berg and Kramer (1979) measured natural water complexing capacity by an ion exchange method.  $MnO_2$  particles are added to the sample, then the sample is titrated with  $Cu^{2+}$ . After each addition of metal ion, an aliquot of the suspension is filtered and the acidified filtrate is analyzed for  $Cu^{2+}$  concentration. The  $MnO_2$  acts as a free metal ion scavenger when it encounters complexes with conditional stability constants smaller than  $10^{10}$  at pH 8, and therefore can be used to monitor the free metal ion species during a titration. Once again, however, a metal ion binding agent is added to a natural water system in this technique without regard for changes in the

complicated equilibria involved. The behavior of 8-hydroxyquinoline solutions and natural water media are simplistically compared in this proposal and no corroborating evidence with another analytical technique is obtained. This complexing capacity measurement also is subject to a systematic error if metal complexes as well as metal ions adsorb to the  $\text{MnO}_2$  particles and the adsorbed complexes are subsequently measured as free metal ions. This event is likely because the adsorptive properties of humic materials are well known in general and have been demonstrated with  $\text{MnO}_2$  (Guy, Chakrabarti & Schramm, 1975).

Abdullah et al. (1976) and Florence and Batley (1976) also made ion exchange binding capacity measurements. These studies used Chelex-100 resin to distinguish strongly and weakly bound metal ions. Figura and McDuffie (1979) compared complexing capacity determinations by Chelex-100 separation to determinations by ASV. They conclude that the two techniques measure different capacities because of the different time scales of the processes. Nygaard and Hill (1979) concur that the fraction of complexes that are Chelex labile is larger than the ASV labile fraction. They point out that should any of the metal complexes slowly dissociate, the slower ion exchange metal binding measurement would detect the slowly freed metal ions. The more rapid ASV measurement would take place before any significant metal complex dissociation. As a result, ASV measured binding capacities would be larger than Chelex-100 capa-

cities. Figura and McDuffie (1979) present a "binding spectrum" representing the species measured by various techniques which range from DPASV measurement of very labile and free metal ions to Chelex-100 measurements of slower dissociating complexes to acid digestion measurement of all metal species including inert complexes.

Potentiometric methods. Ion selective electrode potentiometry (ISE) is an early method of distinguishing free from complexed metal species that is the most straightforward and most used of all the methods available. Ramamoorthy and Kushner (1975) measured the heavy metal binding capacities of river water and of molecular weight fractionated river water with  $\text{Hg}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  ISE. They found that the river water binding capacity was associated with the organic matter in the sample and that the binding capacity of the metal ions followed the order  $\text{Hg}^{2+} > \text{Pb}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+}$ . They also found that most of the binding capacity for all metal ions except  $\text{Cu}^{2+}$  is associated with the smaller molecular weight fractions (<16,000).

Buffle et al. (1977) also found that the metal binding components of fulvic acid are the low molecular weight components. In this study, ISE determinations of metal fulvate stability constants and metal ion binding capacities of two freshwater samples were evaluated by comparison to literature values. Their observations significant to complexing capacity measurements included the detection of two complexing sites and a hysteretic pH effect at  $\text{pH} > 7$  of the

ligand binding properties.

The ISE analytical method has been used by others to characterize some Maine freshwater sources (Giesy, Briesse & Leversee, 1978) and river and well waters (McCrary & Chapman, 1979). McCrary and Chapman (1979) measured the response of copper(II) complexing capacity to variations in pH, alkalinity and complexing agent concentration. They found that, in the absence of strong complexing agents (e.g., EDTA), the complexing capacity is controlled by alkalinity, pH and organic matter concentrations. The formation of copper carbonate and hydroxide species explains the effect of alkalinity and pH on the complexing capacity of the samples.

In spite of its popularity and convenience, ISE potentiometry is not an ideal technique for the measurement of natural water complexing capacity. Ion selective electrode potentiometry has some real limitations in this application. For instance, Sekerka and Lechner (1978) demonstrated a strong pH dependence of solid state Cu(II) electrodes at  $\text{pH} > 4$ . The consequence of this proton sensitivity is the need to carefully maintain a constant pH during a complexing capacity titration. Another adverse effect noted by the authors is that fulvic acid gradually poisons the electrode surface, so the electrode surface must be repolished frequently to expose a clean surface area. The same surface erosion was seen when the electrode was immersed in alkaline solution where the surface is altered by hydrolysis of

copper(II).

McCrary and Chapman (1979) point out that ISE titrations are prone to being performed too quickly. The electrode response, particularly at low copper concentrations, is very slow. They recommend up to one hour equilibration periods between titrant additions at low  $\text{Cu}^{2+}$  levels. Aside from the slow electrode response, they found a 20% additional complexation of copper(II) by fulvic acid occurred over a 24-hour equilibration period. Both of these slow effects indicate that ISE potentiometric titrations can provide erroneously low complexing capacity results by being performed too quickly.

Some other limitations of ISE titrations for the measurement of natural water complexing capacity include the need to introduce electrolyte to insure constant metal ion activity coefficient during the titration, the non-Nernstian behavior of the electrode at low metal ion concentrations, and the high detection limits (ca.  $10^{-6}$  M) relative to natural water metal ion levels. In fact, the detection limit and response of the electrodes may be inadequate to measure a complexing capacity in relatively ligand-free waters.

The need to adjust pH and ionic strength of all solutions titrated by ISE potentiometry is a serious limitation since these adjustments undoubtedly shift the natural equilibria under investigation. Increasing the pH and the solution ionic strength have opposed effects on the metal bind-

ing strength of fulvic acid according to Saar (1980). He found copper(II) fulvate complex formation constants to increase with pH, but decrease with increased ionic strength. Other properties of natural water constituents, like flocculation tendencies and molecule configuration changes, are also pH and ionic strength dependent.

Voltammetric methods. A controversial but appealing analytical method for determining complexing capacity is voltammetric titration. The attractive features of metal ion measurements by anodic stripping voltammetry (ASV) are sufficiently low detection limits (ca.  $10^{-9}$  M) for measuring natural metal ion levels and especially specificity, enabling free and complexed species to be distinguished. Chief disadvantages are the possibility of electrode adsorption of natural ligands, the effect of complex dissociation during the analysis, and the lack of a rigorous understanding of the electrochemical processes during the stripping step of the ASV process. Efforts to resolve these difficulties are deemed worthwhile, however, because of the low detection levels and speciation capability of ASV. A good summary of ASV fundamentals is a review by Copeland and Skogerboe (1974). Schonberger and Pickering (1980) demonstrated the above mentioned effects of pH changes and metal complexation on ASV currents and potentials.

Chau's research group (Chau, Gächter & Lum-Shue-Chan, 1974; Chau & Lum-Shue-Chan, 1974) made an early application of ASV to determine the metal ion binding capacity of natu-



ral waters. The original techniques employed by this group are a foundation for efforts by other researchers (O'Shea & Mancy, 1976; Florence, 1977; O'Shea & Mancy, 1978). Labile complexes and free  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  were measured in acetate buffered lakewater, then the sample was oxidized with  $\text{K}_2\text{S}_2\text{O}_8/\text{H}_2\text{SO}_4$  and the total metal ion concentrations were measured. With Chau's procedure, the metal ion binding capacity can be determined by titrating the sample with metal ion and monitoring the uncomplexed metal concentration by ASV. NCL, PCL and complexing capacity can all be found with this procedure.

Other researchers developed ASV methods. In particular, Florence (1972) recommends ASV application to seawater analysis and Batley and Florence (1976a) developed a metal speciation scheme that uses ASV to differentiate between labile and inert metal complexes in natural water. Unfortunately, voltammetric analysis of natural water has some limitations that are often not considered. Some of the important limitations of voltammetry, and ASV in particular, will be mentioned.

To begin with, the most severe criticism concerns the applicability of static mercury drop techniques like ASV to systems with surface active organic compounds, like lake and river water. Although the effects of surface active compounds were observed early in the use of voltammetry (Hume & Carter, 1972; Brezonik, 1974), more recent observations by Batley and Florence (1976b) and Jacobsen and Lindseth (1976)

suggest that due to the voltammetric artifacts of these compounds, ASV might be inappropriate for natural water analysis. The effects of the surfactants are twofold. The first type of interference results from the electrode surface becoming coated with electroinactive surfactant and the subsequent inhibition of depolarizer (metal ion) diffusion during both the plating and stripping steps. The consequences are depressed or enhanced voltammetric currents and changes in both wave form and stripping potential.

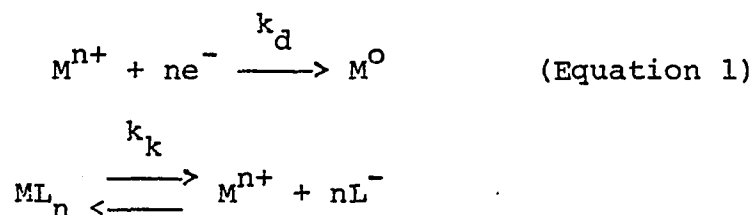
Another consequence of this effect is the possibility of additional complexation of the diffusing depolarizer with the adsorbed ligands. Because of ligand accumulation at the electrode surface, the bulk solution metal-ligand equilibrium is considerably altered in the vicinity of the electrode. Thus, the diffusing metal ion is subjected to complexation as it approaches the electrode surface, which has the effect of depleting the amount of the metal ion reduced and changing the plating and stripping potentials. Stolzberg (1977) suggests that this ligand accumulation process can occur even with surface inactive ligands.

The second interference is the possible appearance of tensammetric waves from non-faradaic adsorption-desorption processes. These waves alter the voltammogram baseline in an irregular fashion and can obscure the metal ion stripping peak by superimposition. A possible way of overcoming this interference is the use of alternate working electrodes. Weber and Cheng (1979), for instance, have reported the

glassy carbon electrode to be free from fulvic acid adsorption interferences. In the analysis of  $\text{Cu}^{2+}$  however, other characteristics of this electrode limit its usefulness (Smart & Weber, 1980).

Another difficulty with stripping voltammetry is the contribution of metal ions from dissociation of very labile complexes to the measurement of free metal ion concentration (Hanck & Dillard, 1977). ASV measures both uncomplexed metal ion concentrations and the concentration of metal complexes that dissociate faster than the plating reaction. These rapid dissociation reactions result in an ASV kinetic current contribution to the diffusion current.

This kinetic current difficulty can be overcome. In the presence of a labile complex, the plating process and dissociation reactions may be expressed as follows:



where  $k_d$  is the faradaic diffusion process rate constant,  $k_k$  is the complex dissociation rate constant, M is the metal ion, and L is the ligand. Shuman and Woodward (1973, 1977) show that the observed ASV current  $i_a$  is the product of the diffusion and kinetic currents:

$$i_a = i_d + i_k \quad \text{(Equation 2)}$$

So, binding capacity determinations by metal ion titrations

would have erroneous endpoints because a smaller quantity of chelating agent than is actually present would be indicated by an enhanced free metal ion determination (the kinetic current contribution). Fortunately,  $i_d$  and  $i_k$  can be distinguished. In the case where there is no  $i_k$ , a plot of  $i_a$  vs. [ligand] has a slope of zero, and conversely, a non-zero slope indicates the contribution of a kinetic current. In this latter case, extrapolating to [ligand] = 0 isolates the diffusion current so titration endpoints can be corrected for kinetic current contributions to reveal the actual metal ion binding capacity (Equation 2). Shuman and Cromer (1979) have measured the copper binding capacity of water-derived fulvic acid using an ASV titration. They obtained a capacity of  $0.9 \times 10^{-5}$  M copper for 15 mg/L fulvic acid at pH 7. They also measured a kinetic current corrected copper fulvate formation constant of  $5 \times 10^5$ .

Ultrafiltration and dialysis methods. Metal speciation and metal ion binding capacity measurements have also been made using separation techniques to distinguish metal species prior to their measurement, rather than direct specific detection with ISE or ASV. The separations are based upon the molecular size difference between the free, uncomplexed metal ion and the large humic-complexed metal species. Both dialysis and ultrafiltration have been used extensively.

Smith (1976) separated estuarine water into 100,000, 50,000, 10,000 and 1,000 molecular weight fractions by ultrafiltration, then titrated to determine the  $\text{Cu}^{2+}$  binding capa-

city of each fraction by Chau's method. The results indicate the smallest fraction had most of the  $\text{Cu}^{2+}$  complexing agents in all cases, except samples with no salinity.

Barsdate (1970), in an early dialysis study, found that lakewater retained  $\text{Co}^{60}$ ,  $\text{Mn}^{54}$ ,  $\text{Zn}^{65}$ ,  $\text{Pb}^{210}$  and  $\text{Cu}^{64}$  when dialyzed against distilled water. In contrast, seawater was less efficient in complexing the radionuclides. Hart and Davies (1977) used dialysis on freshwater samples to define the relative dissolved and colloidal metal concentrations. They point out that membrane surface charge and long dialysis times are the chief disadvantages with this separation technique.

The Hart and Davies (1977) experiment was very similar to an extensive study by Beneš<sup>V</sup>, Gjessing & Steinnes (1976) of the size fraction complexing capacity of brook water for 17 elements using ultracentrifugation, ultrafiltration and ion exchange with neutron activation analysis (NAA). Zunino and Martin (1977) measured the metal binding ability of soil humic materials by immersing a 12,000 molecular weight cut-off dialysis bag containing the ligand in metal ion solution. The metal content of the internal solution was measured at equilibrium by AA and compared to the total metal content of the external dialysis solution to determine the metal binding ability of the ligand.

Beneš<sup>V</sup> and Steinnes (1974, 1975) have speciated the dissolved metal forms and distinguished metal transport mechanisms in both dissolved and particulate phases with in

situ dialysis membranes and neutron activation analysis. Their studies have emphasized the problems associated with manipulating natural water samples prior to metal analysis. In particular, metal losses from storage, filtration and dialysis are demonstrated, and the question of shifting metal speciation equilibria during the analysis is addressed. In situ dialysis is very suitable at natural metal species levels. Back in the lab, dialysis methods can provide complexing capacity measurements with little risk of contaminations or equilibrium disturbance. Dialysis speciation and spectrometric metal analysis also offers a much greater range of metal ions that can be studied by ISE or voltammetric techniques.

### Experimental

#### Materials and Reagents

All solutions were prepared with water that was doubly deionized and distilled from  $\text{KMnO}_4$  solution. Analytical grade reagents were used to prepare all solutions with three exceptions. Ultrex (Ventron Corp., Beverly, Mass.) nitric acid was used to acidify natural water samples, dialysis solutions, and standard metal ion solutions. The metal ion standard solutions were all prepared from Fisher 1000 ppm Atomic Absorption solutions. Finally, all SFA solutions were prepared from soil-derived fulvic acid isolated and characterized by Weber and Wilson (1975).

Glass, polycarbonate, polypropylene and polyethylene

apparatus and vessels were cleaned by soaking in 10%  $\text{HNO}_3$  followed by rinses with distilled water. All metal ion solutions were prepared in polypropylene volumetric flasks using disposable polycarbonate tipped pipets. Dialyses and sample storage were carried out in polypropylene bottles. Natural water filtration was accomplished with the polycarbonate filter assembly described in Chapter 3, but a 142 mm Teflon and plexiglass filter support (Nuclepore Corp., Pleasanton, CA.) was substituted for the 47 mm polycarbonate support.

The 1000 molecular weight cut-off (m.w.c.o.) Spectra/Por 6 dialysis bags (Spectrum Industries, Los Angeles, CA.) were cleaned prior to use with a procedure recommended by Guy (1976). The bags are rinsed and soaked in warm water to remove the preservatives, then they were soaked in 0.1%  $\text{Na}_2\text{S}$  solution at about 60°C for 15 min. The bags were then rinsed with warm water and soaked in 3%  $\text{H}_2\text{SO}_4$  at about 60°C for 5 min. The bags were then rinsed in warm distilled water again, and were finally stored in distilled water until needed.

#### Natural Water Sampling

Water samples were taken from seven locations in southeastern New Hampshire that are designated on the map in Figure 8. Samples were taken from the Oyster River (O), the Exeter River (E), the Lamprey River (L), the Durham reservoir (D), and the Portsmouth reservoir (P), as representatives of local drinking water supplies. A sample of Barrington

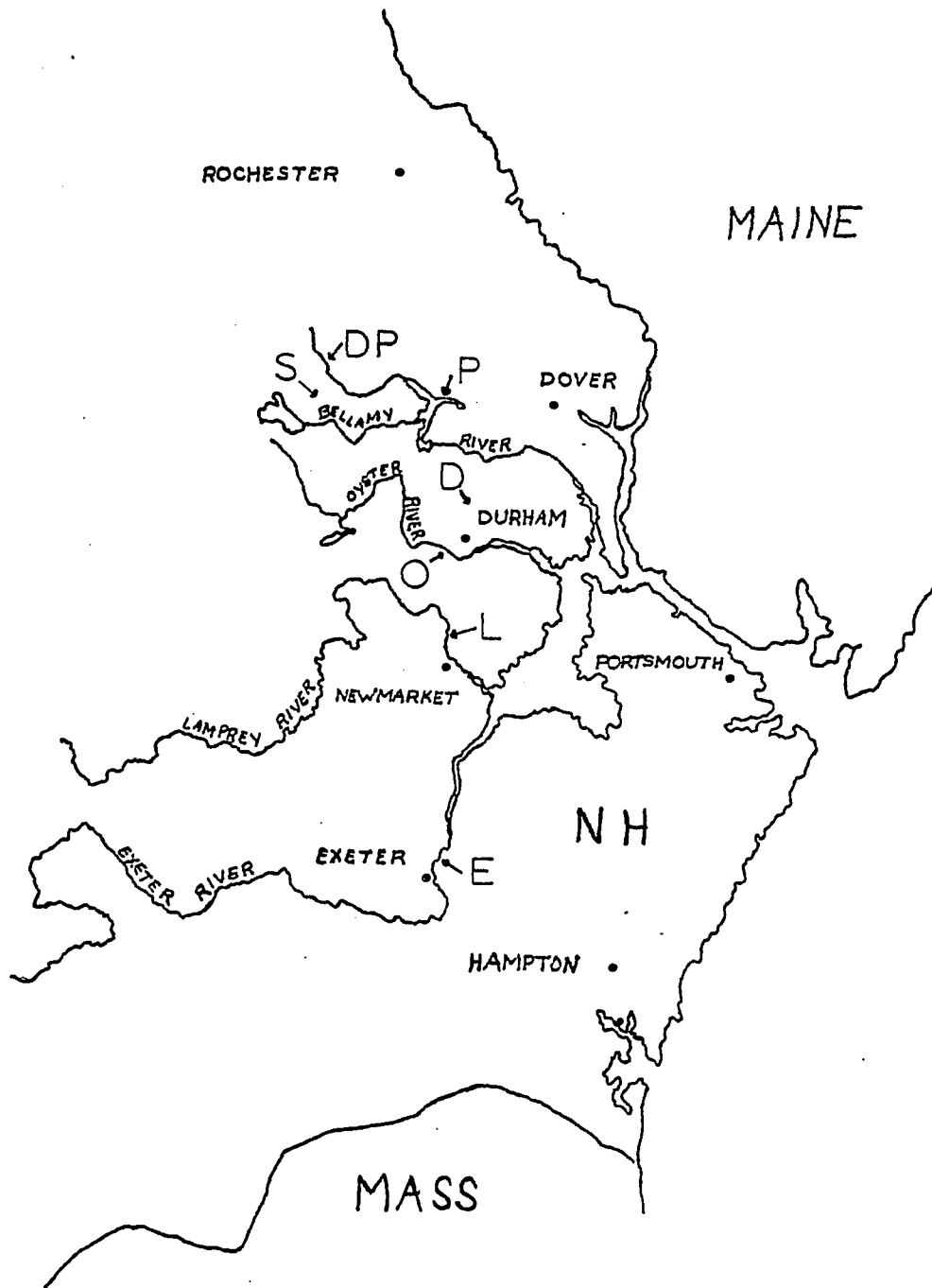


Figure 8. Map of Southeastern New Hampshire Identifying the Natural Freshwater Sample Sites



ton swamp water (S) and Drew Pond (DP) water were taken for comparison with the drinking water supply samples.

The water samples were all obtained by submersing acid-washed polypropylene bottles six inches below the surface then releasing the bottle cap. The bottles were rinsed with sample before an aliquot was collected. Approximately 10L of sample was taken at each site. The samples were taken back to the laboratory and immediately filtered through 0.45  $\mu\text{m}$  polycarbonate membranes using the filter apparatus previously described. The samples were packed in ice during the filtration and were finally stored in polypropylene bottles at 4°C.

#### Characterization of the Water Samples

Several properties of each natural water sample were determined. Except for the color, fluorescence and PCL measurements, all characterization procedures were taken from Standard Methods for the Examination of Water and Wastewater (1975).

A. Natural water sample pH measurements were made with an Orion 701A electrometer (Orion Research Inc., Cambridge, Mass.) and a Corning model 476050 combination pH electrode (Corning Science Products, Medfield, Mass.).

B. Alkalinity determinations were made by titrating each sample with 0.0288 M HCl to pH 3.7 in triplicate, with the same apparatus used to measure pH. Alkalinity was obtained from the titration endpoint.

C. Dissolved organic carbon (DOC) was determined with a Sybron/Barnstead PHOTOchem Organic Carbon analyzer (Barn-

stead Company, Boston, Mass.), using 0.21254 g/L KHP (100 mg/L C) as a reference standard. At least triple replicate measurements were made.

D. Water sample hardness was measured by titrating with 0.01003 M EDTA to an Eriochrome Black endpoint, in triplicate.

E. All metal ion levels were measured by atomic absorption spectrometry (AAS). Iron measurements were made on a Techtron AA5 spectrometer (Varian Corp., Melbourne, Australia) with an air-acetylene flame. Lead, copper and cadmium measurements were made with an IL351 spectrometer and IL555 atomization programmer using pyrolytic graphite furnaces (Instrumentation Laboratory Inc., Wilmington, Mass.).

F. Color measurements were made with a Cary 14 double beam spectrophotometer (Applied Physics Corp., Monrovia, CA.). Using distilled water as a reference solution, color was determined by scanning the 300-260 nm spectrum of each sample at the natural pH and at pH 7.6. The reported color is their 260 nm absorbance in a 1 cm path length cell at pH 7.6 compared to a calibration curve produced with a dilution series of pH 7.6 SFA standard solutions.

G. Fluorescence measurements were made with a Perkin-Elmer 204 spectrofluorometer and a Perkin-Elmer 150 Xenon lamp power supply (Perkin-Elmer Corp., Norwalk, Conn.). Fluorescence emissions were determined against distilled H<sub>2</sub>O using 1 cm<sup>2</sup> quartz sample cells. Samples were excited with 350 nm light and 435 nm emission was measured. Fluorescence

measurements of all samples and standard SFA solutions were made at pH 7.6, using the same calibration procedure as in the color measurements.

H. Conductance was measured at 25.0°C with a YSI 31 conductance bridge (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) and a Pt black conductance cell having a cell constant of  $0.260 \text{ cm}^{-1}$ . KCl standard solutions were used to determine the cell constant.

I. Copper(II), cadmium(II) and lead(II) potential complexing level (PCL) measurements were made by the dialysis/AA technique described in the next section.

#### Complexing Capacity and PCL Measurements

Two techniques were used for complexing capacity titrations of soil-derived fulvic acid. The first involved titrating samples while they were dialyzing against a small amount of electrolyte solution. The dialyzable (free) metal ion levels are measured after every addition of titrant. The second method involved titrating fulvic acid with  $\text{Cu}^{2+}$  and monitoring uncomplexed metal ion by ion selective electrode potentiometry (ISE). In both techniques, complexing capacity results were obtained for SFA from plots of free vs. total metal ion levels. The same experimental procedure using natural water samples yields PCL's since copper(II) and cadmium(II) were present (NCL) in all the samples.

Dialysis titration procedure. The dialysis titration technique involved immersing a dialysis bag with 80 mL 0.001

$\text{M KNO}_3$  in 2L of sample solution. The internal dialysis solution (diffusate solution) pH was adjusted to match the external (retentate) dialysis solution. Dialysis was performed at room temperature, with constant stirring for an equilibration period of 48 hours between titrant additions. After this equilibration period, 5 mL aliquots of dialysis retentate and diffusate solutions were taken and acidified with 50  $\mu\text{L}$  reagent  $\text{HNO}_3$ . Metal ion concentration measurements were performed by flame and flameless AAS using a calibration curve of standard solutions.

This procedure was used to measure the complexing capacity of 6.25  $\mu\text{M}$  EDTA, 0.001  $\text{M KNO}_3$ , 15.5  $\mu\text{M}$  SFA, and the PCL of the natural water samples. Five copper(II)-SFA dialyses were conducted in an experimental design that allowed a comparison of dialysis titration results with ion selective electrode potentiometric results in a paired t-test. A brief description of the paired t-test will be given so that the experimental results can be discussed in the context of the abilities and limitations of this statistical analysis.

Paired t-test procedure. In the experimental design for the paired t-test (Natrella, 1963, p. 3-38), the copper complexing capacities of each of five SFA solutions was measured by both dialysis/AA and ISE potentiometric titration in a random order. Differences between the results of the two techniques are calculated for each solution, then the average ( $\bar{y}$ ) and standard deviation (s) of these differences is calculated. These parameters are used to calculate the

ratio of the mean to the number weighted standard deviation using Equation 3:

$$t = \bar{y} \sqrt{n}/s \quad (\text{Equation 3})$$

In Equation 3,  $t$  is the student's  $t$ -statistic,  $\bar{y}$  is the average difference between pairs of measured complexing capacities,  $s$  is the standard deviation of this mean, and  $n$  is the number of pairs.

The calculated  $t$  is compared to tabulated values (Natrella, 1963) at the desired confidence level and for the appropriate degree of freedom. If the calculated probability ( $t$ ) exceeds the tabulated values, one may conclude with the indicated level of certainty that the two methods yield different results on the average. A calculated  $t$  smaller than the tabulated value means that the hypothesis of no difference in the average dialysis results and the average ISE results cannot be rejected at the stated confidence level.

Copper ion selective electrode potentiometric titrations. A paired  $t$ -test of dialysis titration complexing capacities and copper ISE titration complexing capacities was conducted to determine whether the two titration techniques provide the same results. Five  $15.5 \mu\text{M}$  SFA solutions were prepared; two at pH 6, two at pH 7, and one at pH 5, and the complexing capacities of each were determined by both methods. The experimental design is given in Table 9.

Calibration titration curves ( $0.01 \text{ M KNO}_3$ ) were taken

Table 9. Summary of ISE Titrations of 15.5  $\mu\text{M}$  SFA (Paired t-Test Study)

<u>Titrated Solution</u>	<u>Titrant</u>	<u>Complexing Capacity (<math>\mu\text{M}</math>)</u>	<u>Titration Slope</u>
A. pH 5	Cu	22.6	.884
		23.5	.918
		22.0	.695
B. pH 6	Cu	27.8	.867
		23.8	.856
		28.7	.838
C. pH 6	Cu	25.9	.999
		30.1	.922
		22.2	.946
D. pH 7	Cu	49.5	.458
		38.1	.405
		44.0	.498
E. pH 7	Cu	43.5	.784
		42.7	.761
		44.5	.587

three times daily to measure the ISE response to  $\text{Cu}^{2+}$ . The calibration curves were run at exactly the same pH as the SFA titrations since constant pH is critical to the electrode response at  $\text{pH} > 4$  (Sekerka & Lechner, 1978). All SFA titrations were carried out in  $0.01 \text{ M KNO}_3$  to maintain a constant ionic strength throughout the experiment and the titrations were performed at  $25.0^\circ\text{C}$  with light excluded from the titration vessel. The electrode required frequent polishing during the titration of high pH solutions in order to retain a Nernstian response. Electrode response times were slower at pH 7 than at pH 5, but 5 min. to 1 hr. equilibration periods were used between additions at all pH values.

#### Dialysis Membrane Permeability

The 1000 m.w.c.o. dialysis membrane permeability to  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and SFA was determined by sampling another series of metal ion-SFA dialysis diffusate and retentate solutions as a function of time. The experiments listed in Table 10 were used for these permeability studies, where 2 L of either 10 mg/L SFA or  $0.001 \text{ M KNO}_3$  was dialyzed against 80 mLs  $0.001 \text{ M KNO}_3$ . The metal ion concentration of all dialyses was 2 mg/L. The pH of all internal and external dialysis solutions was adjusted to the appropriate level (Table 10) with minute volumes of  $0.01 \text{ M KOH}$ .

In these permeation experiments, the membrane diffusion rate of the metal ion and organic matter was monitored by taking aliquots of internal and external dialysis solutions

Table 10. Experiments Used to Determine Dialysis Membrane  
Permeability to SFA,  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$

SOLUTION			
	<u>Retentate</u>	vs. <u>Diffusate</u>	<u>pH</u> <u>M<sup>2+</sup></u>
I.	15.5 $\mu\text{M}$ SFA	0.001 $\text{M}$ $\text{KNO}_3$	
	A		5      Cu
	B		6      Cu
	C		7      Cu
	D		5      Cd
	E		6      Cd
	F		7      Cd
II.	0.001 $\text{M}$ $\text{KNO}_3$	0.001 $\text{M}$ $\text{KNO}_3$	
	G		5      Cu
	H		6      Cu
	I		7      Cu
	J		5      Cd
	K		6      Cd
	L		7      Cd
III.	62.0 $\mu\text{M}$ SFA	0.001 $\text{M}$ $\text{KNO}_3$	5      -



periodically. Soil-derived fulvic acid diffusion was monitored by taking 10 mL solution aliquots, raising their pH to 7.6, then measuring their 260 nm color and 435 nm fluorescence emission compared to a pH 7.6 SFA calibration curve. To determine metal ion diffusivity, 5 mL aliquots of dialysis retentate and diffusate solution were taken, acidified with 50  $\mu$ L reagent  $\text{HNO}_3$ , and analyzed for metal ion concentration by flame AAS.

The third experiment in Table 10 was a dialysis of 62  $\mu\text{M}$  (40 ppm) SFA against 0.001 M  $\text{KNO}_3$  at pH 5 for 36 days with no metal ions added. At the end of this dialysis period the color, fluorescence and copper(II) binding capacity permeation of the membrane was measured. Color and fluorescence were measured the same way as in experiments I and II in Table 10. Copper(II) binding capacities of the dialysis retentate and diffusate solutions were determined in a fluorometric titration at pH 5, where after each addition of  $\text{Cu}^{2+}$  and pH adjustment the 435 nm fluorescence intensity of the dialysis solution was measured. Distilled water was the reference solution. Plots of fluorescence intensity vs. total  $\text{Cu}^{2+}$  added were used to calculate the intersection of the upper and lower titration curve branches. The total  $\text{Cu}^{2+}$  added value of this intersection is the binding capacity of the titration solution.

## Results and Discussion

### Dialysis Membrane Permeability

The validity of natural water complexing capacity determinations by dialysis titration rests on two requirements. First, the dialysis membrane should effectively exclude complexed metal ion and uncomplexed ligand from the internal solution. As already pointed out, the dialysis internal solution metal ion concentration is assumed to be free metal ion, so the presence of complexed metal in the internal solution causes an underestimation of the ligands complexing capacity. Similarly, free ligand that passes the membrane is not available for complexation in the retentate solution. This also causes an underestimation of the complexing capacity.

As a second requirement, the uncomplexed metal ion diffusion through the dialysis membrane must be unhindered so that internal (diffusate) and external (retentate) titration solution uncomplexed metal ion levels are the same at equilibrium. This is necessary because the internal dialysis solution metal ion concentration is taken as the equilibrium "free" metal ion concentration in dialysis complexing capacity determinations.

Both of these requirements were tested in SFA and  $\text{KNO}_3$  (control) dialyses (Table 10).

Experimental control  $\text{KNO}_3$  dialyses. The  $\text{KNO}_3$  vs.  $\text{KNO}_3$  dialysis (solutions G-L in Table 10) are experimental blank dialyses. That is, these diffusate and retentate solution

color and fluorescence measurements are background levels for comparison with the SFA dialysis solution measurements. Metal ion diffusion that is independent of the influence of SFA also can be determined in these experiments. A discussion of the color, fluorescence and metal ion concentrations in the  $\text{KNO}_3$  control dialyses follows.

The internal and external metal ion levels of the  $\text{KNO}_3$  control solutions show no time dependence on the time scale of days (Figure 9). Metal ion diffusion is apparently rapid since  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  levels in all the control dialyses were unchanged from 1 to 27 days of dialysis. The diffusion appears to be pH independent. In the absence of SFA at least metal ion diffusion through the membranes seems unhindered.

The color (260 nm absorbance at pH 7.6 expressed in mg/L SFA) of all the diffusate and retentate solutions gradually increases over the period of a dialysis titration (Figure 10). The increase is more evident in the diffusate solutions due to their smaller volumes. Moreover, the color increases seem pH dependent: by the 27th day of dialysis the respective retentate and diffusate solution colors (mg/L as SFA) were 3.3 and 3.4 for pH 5, 1.7 and 3.5 for pH 6, and 2.0 and 1.8 for pH 7 copper(II) dialyses (solutions G, H and I in Table 10). The color appearance in the cadmium(II) dialyses behaved similarly (solutions J, K, L in Table 10).

Fluorescence measurements of the  $\text{KNO}_3$  control dialysis solutions show no change as a function of time on the exper-

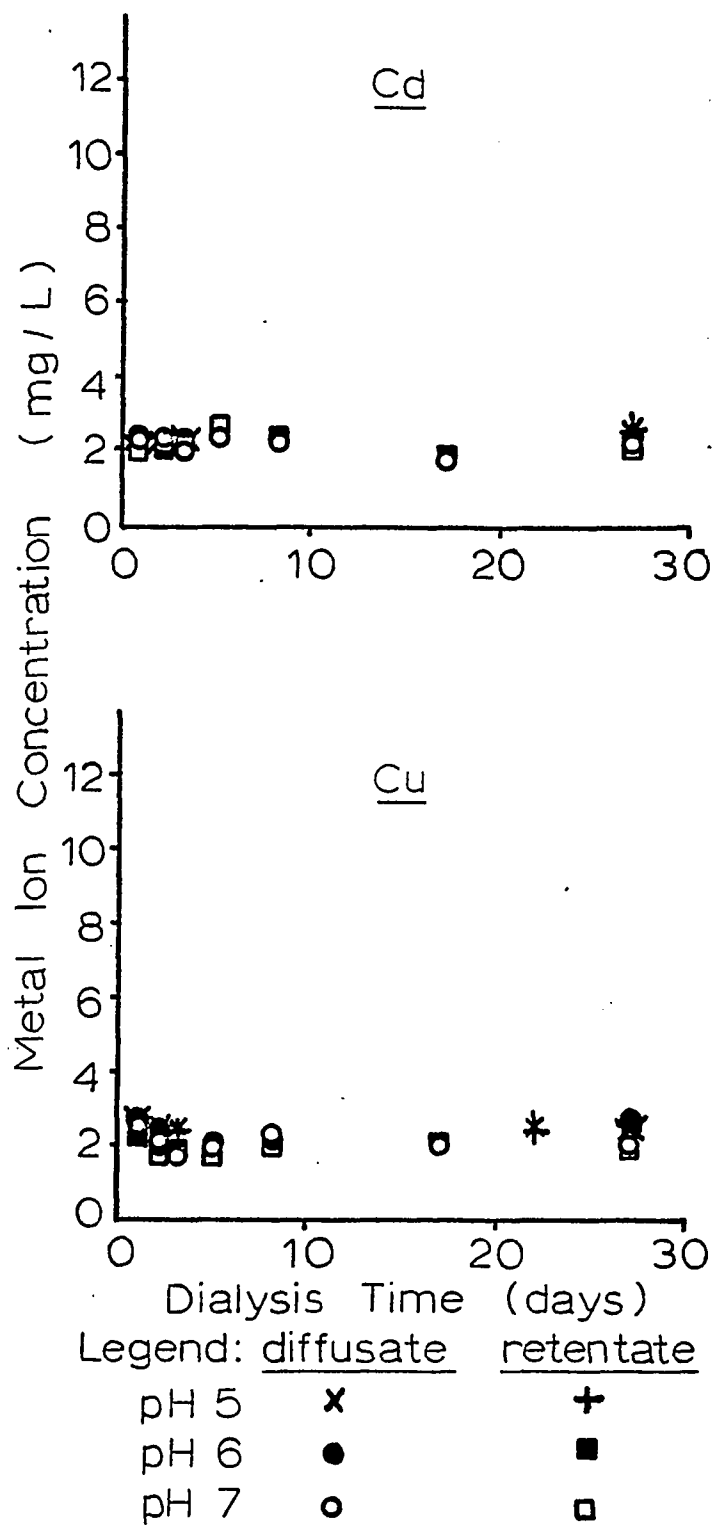


Figure 9.  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  Diffusion as a Function of Time in 0.001 M  $\text{KNO}_3$  vs. 0.001 M  $\text{KNO}_3$  Dialyses at pH 5, 6 and 7

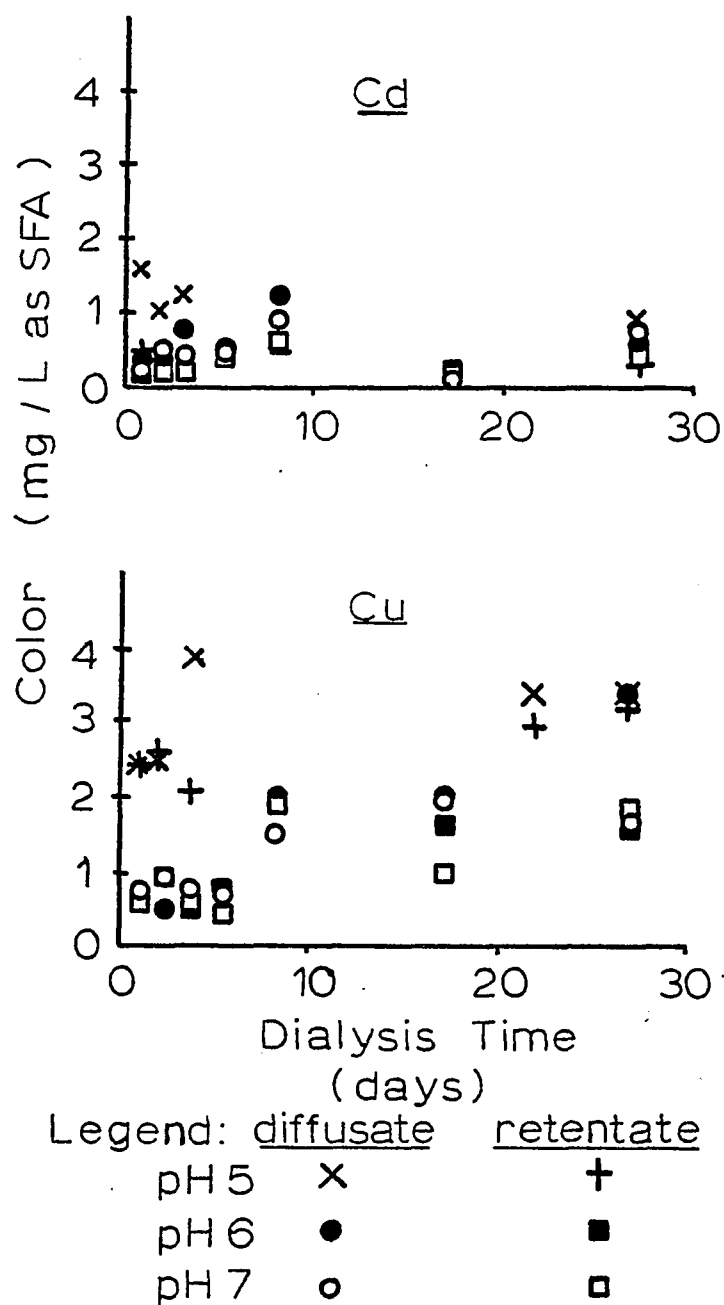


Figure 10. Solution Color as a Function of Time in 0.001 M  $\text{KNO}_3$  vs. 0.001 M  $\text{KNO}_3$  Dialyses with  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  at pH 5, 6 and 7

imental time scale, however (Figure 11). The material responsible for the gradually increasing UV absorbance in the dialysis control solutions appears to have very weak fluorescence properties since the  $\text{KNO}_3$  blank dialysis solutions all have very small (0.1 to 0.4 mg/L as SFA) fluorescence emissions. The fluorescence of the  $\text{KNO}_3$  solutions shows no pH dependence.

The cellulose membranes are undoubtedly the source of the UV absorbing material that is seen in the  $\text{KNO}_3$  dialysis solutions. Flameless AA measurements of membrane wash solutions reveal that small amounts of copper (ca. 50  $\mu\text{g/L}$  after two weeks) also leach from the membranes. The amount of copper extracted from the membrane is greater for 0.001  $\text{M}$   $\text{HNO}_3$  wash solutions than for 0.001  $\text{M}$  acetate buffer (pH 6) wash solutions or distilled water, demonstrating the same pH trend as the leaching of organic matter. Considering the insolubility of the cellulose membranes, the most likely contaminant is a pore size restricting additive in the Spectra/Por 6 1000 m.w.c.o. membranes.

There are some possible adverse effects of these background properties of the membranes. First, the sulfuric acid rinse step of the membrane cleaning procedure must be performed reproducibly on different batches of membranes. Since the contaminant material is leached most effectively by acid solutions, and since this material may be the pore size restricting additive, a significant membrane pore size difference could result between batches of membranes cleaned

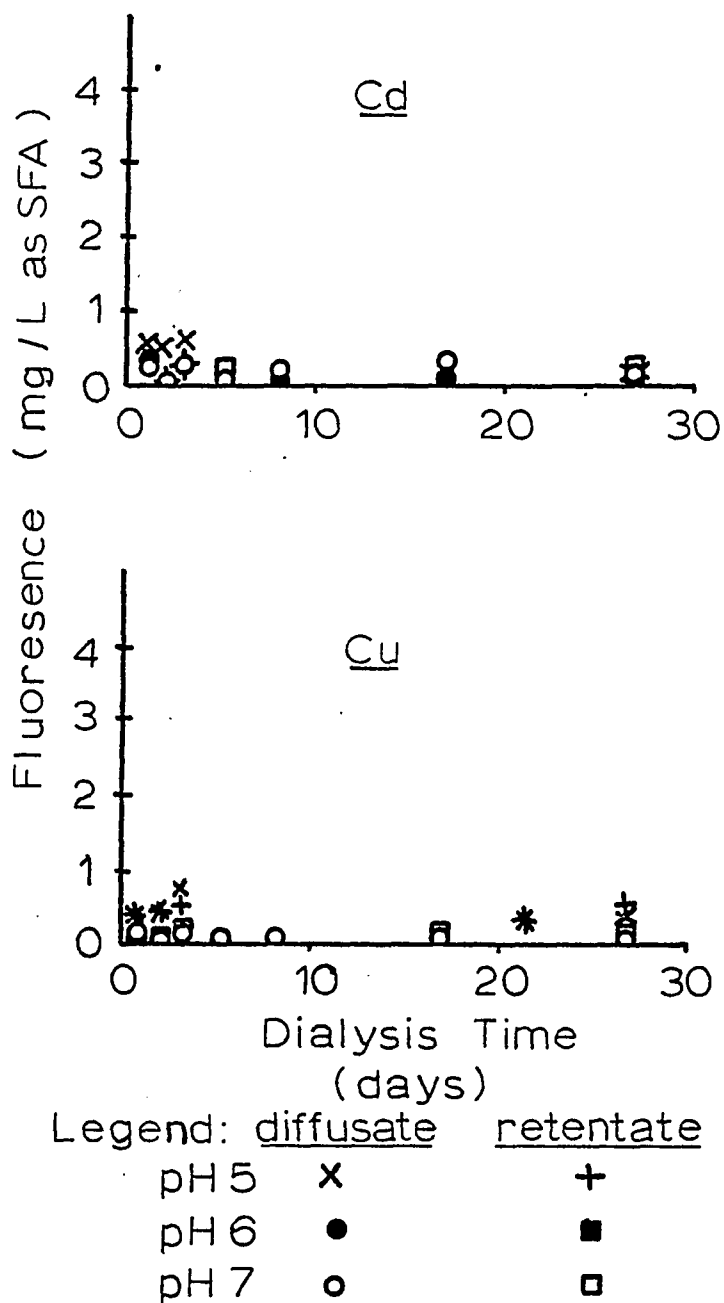


Figure 11. Solution Fluorescence as a Function of Time in 0.001 M  $\text{KNO}_3$  vs. 0.001 M  $\text{KNO}_3$  Dialyses with  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  at pH 5, 6 and 7

in different concentrations of acid, at different temperatures, or for different lengths of time. The result would be poor reproducibility in the membrane separation ability. Second, differences in the loss of this pore size adjusting material in dialysis of different pH would introduce a systematic error in dialysis results that was inversely proportional to pH.

Using this background color and fluorescence data to correct the SFA dialysis color and fluorescence data serves to isolate SFA permeability properties of the dialysis membranes from the undesired properties just described.

Dialyses of  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  in SFA against  $\text{KNO}_3$ . The permeability of Spectra/Por 6 1000 m.w.c.o. dialysis membranes to SFA diffusion was determined from a series of dialyses of 15.5  $\mu\text{M}$  SFA vs. 0.001  $\text{M}$   $\text{KNO}_3$  (solutions A-F in Table 10). The dialysis retentate and diffusate solution color and fluorescence was monitored as a function of time, just as in the  $\text{KNO}_3$  control experiments. Figures 12 and 13 have the color and fluorescence measurements of the copper (II)-SFA and cadmium(II)-SFA dialyses, adjusted to correct for background color (Figure 10) and fluorescence (Figure 11) intensities, plotted against time. The point for point subtraction of  $\text{KNO}_3$  dialysis color and fluorescence intensities from the SFA measurements isolates the contribution of SFA from that of the dialysis membrane material which gradually appears in solution, as previously mentioned.

Diffusate solution color increases up to ca. 15 days



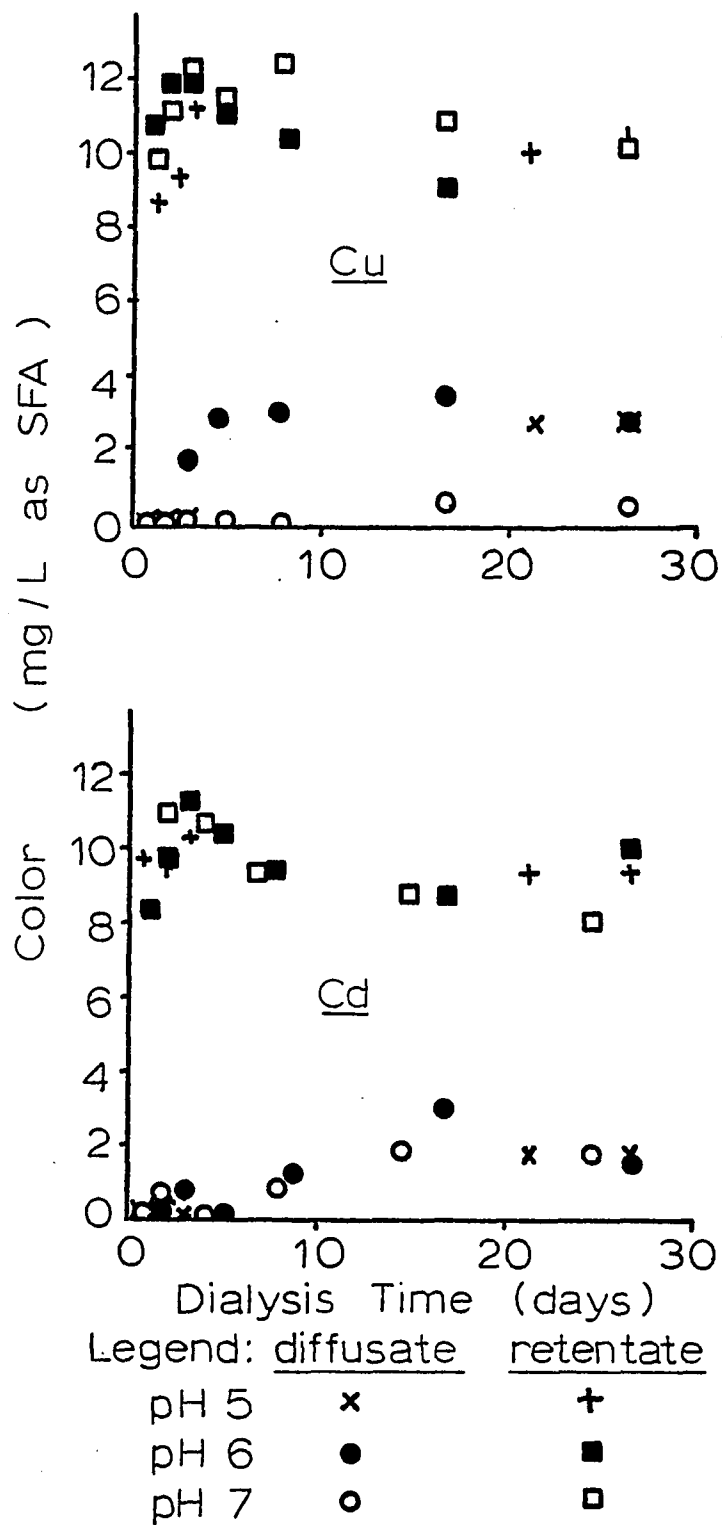
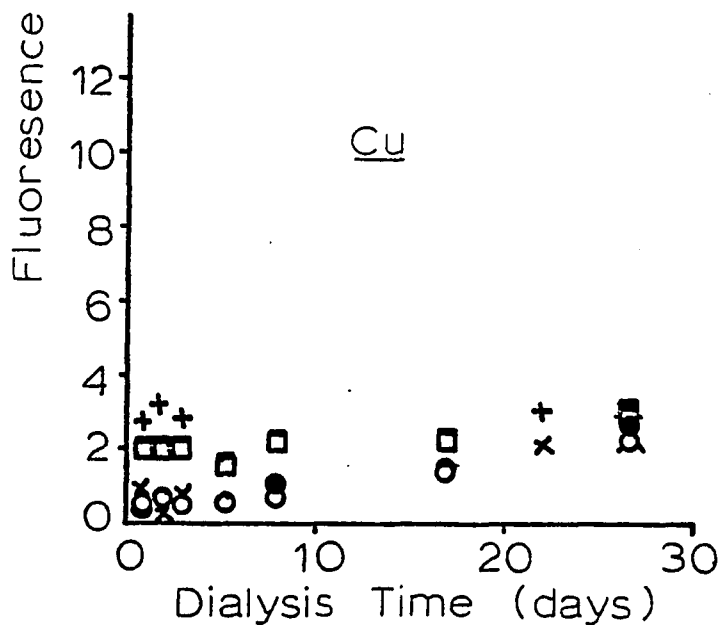
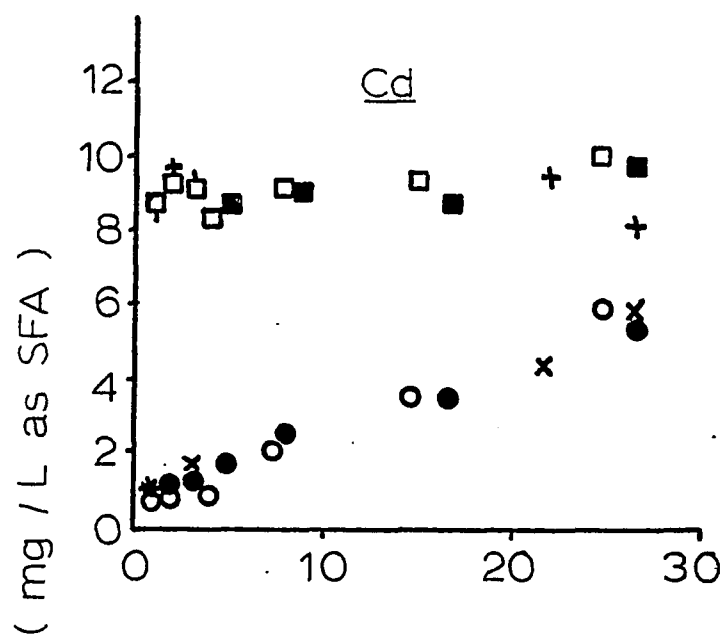


Figure 12. Background Corrected Solution Color as a Function of Time in 15.5  $\mu\text{M}$  SFA vs. 0.001  $\text{M}$   $\text{KNO}_3$  Dialyses with  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  at pH 5, 6 and 7



Dialysis Time (days)

Legend: diffusate      retentate

pH 5	x	+
pH 6	●	■
pH 7	○	□

Figure 13. Background Corrected Solution Fluorescence as a Function of Time in 15.5  $\mu\text{M}$  SFA vs. 0.001  $\text{M}$   $\text{KNO}_3$  Dialyses with  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  at pH 5, 6 and 7

of dialysis before achieving a constant value in all the SFA dialyses (Figure 12). The trend is pH dependent in the copper(II) dialyses where diffusate color rises to ca. 30% of the retentate solution color (11 mg/L as SFA) at pH 5, ca. 30% at pH 6, and less than 5% at pH 7. This pH dependence is much less distinct for the cadmium(II) dialyses. At all pH value the cadmium(II) dialysis diffusate color rises to only ca. 20% of the retentate solution color (10 mg/L as SFA).

The background corrected SFA dialysis fluorescence data shows no pH dependence (Figure 13), possibly due to the small intensities in the copper(II) dialyses and weak pH influences in the cadmium(II) dialyses. In all the SFA dialyses, diffusate solution fluorescence intensities increased gradually over the dialysis period, while the retentate solution intensities remained essentially unchanged throughout. The retentate solution loss of organic matter to the diffusate solution is unnoticed because of the large difference in volumes of these solutions (80/2000 mL). On average the copper(II) dialysis fluorescence diffusion rate was 0.05 mg/L/day. In the cadmium(II) dialyses, the fluorescence increase averaged 0.21 mg/L/day overall.

The fluorescence data in Figure 13 show an important metal ion dependence. Saar (1980) studied spectral properties of metal ion-SFA complexes and showed that the 435 nm fluorescence of SFA, but not its UV absorbance (color), is quenched in direct proportion to its complexation of

$\text{Cu}^{2+}$ . The copper(II) dialysis fluorescence data then is a measure of uncomplexed SFA. SFA complexation by  $\text{Cd}^{2+}$  has no effect on SFA fluorescence (Saar, 1980), and therefore no free ligand information can be obtained from the cadmium (II) dialysis solution fluorescence.

Figure 14 shows a limited pH dependence of metal ion diffusion in the SFA dialyses. Reflecting the greater metal binding ability of SFA at higher pH, there are smaller cadmium(II) and copper(II) diffusate concentrations at pH 7 than at pH 5. This metal ion and SFA diffusion study shows the relative extents of cadmium(II) and copper(II) complexation by SFA in the equilibrium diffusate concentrations of the two metal ions. At pH 7 the free  $\text{Cd}^{2+}$  levels are approximately twice the comparable free  $\text{Cu}^{2+}$  concentrations. Metal ion diffusate solution concentrations do not gradually increase with time. Unlike color and fluorescence intensities, metal ion levels reach equilibrium across the dialysis membrane rapidly, just as they did in the  $\text{KNO}_3$  vs.  $\text{KNO}_3$  control dialyses.

SFA binding capacity diffusion. The copper(II) fluorescence data in Figure 13 shows that some SFA binding material gradually permeates the dialysis membrane. The permeation is governed by a diffusion equation:

$$\text{solute transfer rate} = \frac{DS}{l}(C_1 - C_2) \quad (\text{Equation 4})$$

where D is the diffusivity of the solute, S is the membrane area, l is the membrane thickness, and  $C_1 - C_2$  is the differ-

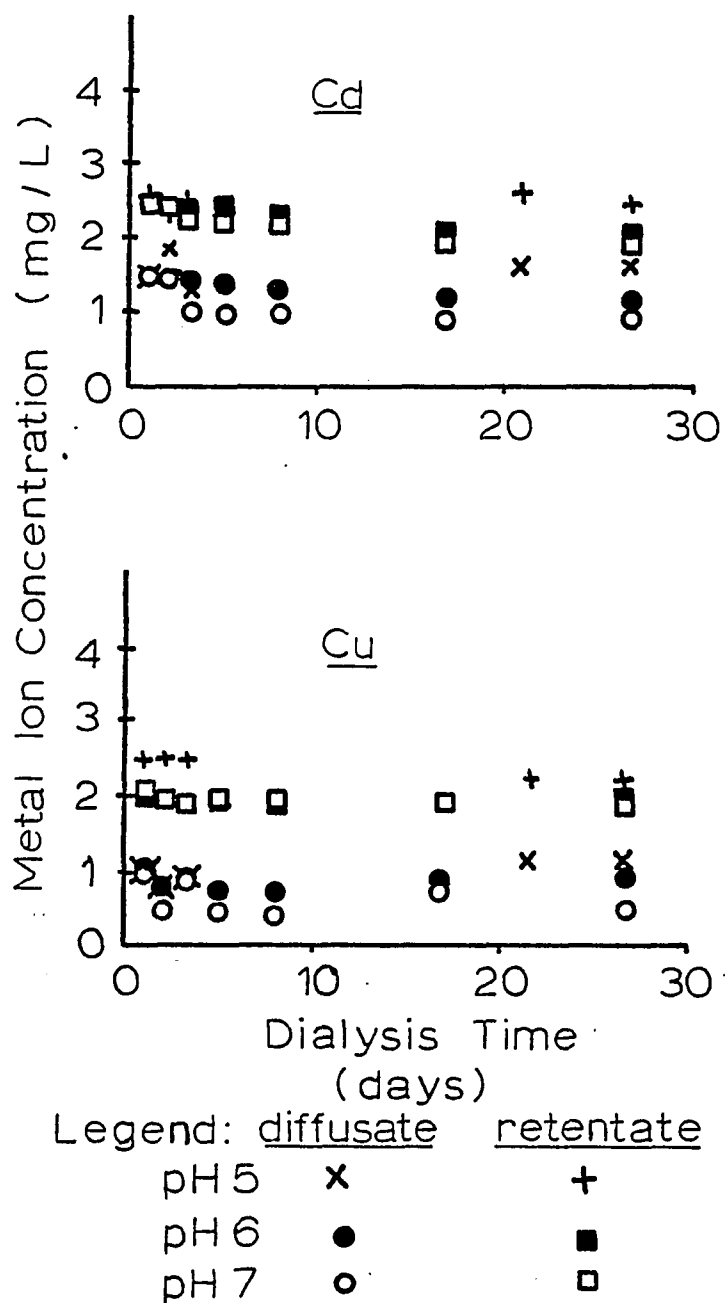


Figure 14.  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  Diffusion as a Function of Time in  $15.5 \mu\text{M}$  SFA vs.  $0.001 \text{ M}$   $\text{KNO}_3$  Dialyses at pH 5, 6 and 7.

ence in solute concentration across the membrane (Hwang & Kammermeyer, 1975). This equation indicates that the binding capacity diffusion rate would be greatest at the beginning of a metal ion dialysis titration when the uncomplexed ligand concentration gradient across the dialysis membrane is greatest. The binding capacity retention of the dialysis membrane can be estimated from diffusate solution color measurements at the end of the titration (Figure 12).

Because of the pore size of the membranes, the organic compounds that diffuse through the membrane in SFA dialyses have average molecular weights less than 1000. Sephadex size separations with SFA indicate that it is indeed composed of two distinct groups of molecules based upon size and metal binding ability. Templeton and Chasteen (in press) found that Sephadex fractionation produced two different size components of SFA. The large molecules (3800-4100 m.w.) constitute 91% by weight of the SFA while the remaining 9% are small molecules (280-310 m.w.). A study of the  $\text{VO}^{2+}$  complexing properties of each fraction revealed that most of the strong binding sites and the bulk of all vanadyl ion binding ability in the unfractionated SFA was associated with the large molecule fraction.

The loss of binding capacities in the dialysis studies represents a limitation to the usefulness of this technique, and the Templeton and Chasteen (in press) results suggest that the dialysis membrane failure to retain metal

ion binding capacity is at worst 9% of the total capacity. This fraction is the binding capacity of the small molecule component of SFA and represents a conservative error estimate since the small molecules are less effective metal binding agents than the large molecule portion of SFA. Indeed, in an ultrafiltration study using SFA, Saar (1980) also reports 5-10% copper binding capacity permeation using 500 m.w.c.o. membranes.

Measurement of the binding capacity that permeates 1000 m.w.c.o. dialysis membranes from 15.5  $\mu\text{M}$  SFA solutions is difficult owing to the small binding capacity to be determined and the insensitivity of complexing capacity measurements. A fluorometric titration, where fluorescence quenching by  $\text{Cu}^{2+}$  is monitored, was performed on 62  $\mu\text{M}$  SFA, pH 5, dialysis retentate and diffusate solutions after 36 days of dialysis. The diffusate concentration of binding capacity will be large enough to measure by raising the retentate solution SFA concentration. The raised SFA concentration and extended dialysis period both act to increase the extent of  $\text{Cu}^{2+}$  binding capacity diffusion over what is expected in a 15.5  $\mu\text{M}$  SFA dialysis titration during a shorter period. Also, less binding capacity diffusion is expected in an actual titration because the amount of diffusable organic matter is reduced by complexation with metal ion after every addition of titrant solution. As a result, the binding capacity diffusion in the 62  $\mu\text{M}$  SFA dialysis (experiment III in Table 10) is a conservative,

worst-case estimate of complexing capacity errors.

The diffusate to retentate fluorescence ratio (pH 7.6) of the 62  $\mu\text{M}$  SFA dialysis was 9/40. The color ratio of these solutions was 3.5/35, indicating that the material diffusing through the dialysis membrane had greater fluorescence than UV absorbing tendency. The color measurement indicates that 10% of the total organic matter permeated the dialysis membrane. This measurement was in agreement with the fluorescence titration results, where 8 and 10%  $\text{Cu}^{2+}$  binding capacity diffusion were measured in two trials.

The Sephadex fractionation (Templeton & Chasteen, in press), the ultrafiltration (Saar, 1980) and the dialysis studies (this work) all suggest that at worst, 10% of metal ion binding capacity by SFA will not be retained by the 1000 m.w.c.o. dialysis membrane. This lost binding capacity will result in underestimation of metal complexing capacity determination by the dialysis titration method used in this study.

#### Dialysis/AA Complexing Capacity Measurements

Having established the metal ion and humic material diffusion properties with the 1000 m.w.c.o. membranes, the next step in evaluating complexing capacity measurements by dialysis/AA was to perform a series of titrations. To establish optimum titration procedures and the performance of the dialysis titration technique in systems better understood than natural environmental systems, the complexing capacities of EDTA and SFA solutions were measured prior to natural water sample measurements. The progression of



titration experiments from EDTA to SFA to natural water is one of increasing complication. While the results from these three systems are not directly comparable, some distinction between the abilities of the measurement technique and the complications of the natural water matrix can be made based upon the results from the simpler systems.

All the complexing capacity titration data is obtained and evaluated in the same way. Sequential additions of metal ion are made to the ligand solution, then free (uncomplexed) metal ion concentration and total (uncomplexed and bound) metal ion concentration are measured after an appropriate equilibration period. Total metal ion concentrations are measured rather than calculated to distinguish the amount of metal ion added from the amount actually available for coordination, should any metal ion be lost from solution by wall effects, precipitation, etc. In the dialysis technique, the internal dialysis bag (diffusate) solution metal ions are considered free, and the external (retentate) solution metal ions are both free and coordinated (total). The retentate to diffusate solution volume ratio is 2000/80 mL.

Each titration curve obtained has two branches: the lower branch of early data points, which has essentially zero slope, and the upper branch of data after the titration endpoint, which is markedly sloped. In each titration, the x-axis intercept of the upper branch, expressed in  $\mu\text{M}$  total metal ion, is defined as the PCL. For EDTA and SFA, the PCL

is the complexing capacity since they have no  $\text{Cu}^{2+}$  or  $\text{Cd}^{2+}$  NCL. The x-axis intercept is calculated from a linear least squares fitted line through the upper titration branch data.

EDTA titrations. Stability constants of the EDTA 1:1 complexes are  $10^{16.5}$  for  $\text{Cd}^{2+}$  and  $10^{18.8}$  for  $\text{Cu}^{2+}$  (Flaschka, 1964). As a result, EDTA complexes both metal ions strongly enough to give a relatively sharp dialysis titration end-point from which a complexing capacity and a metal binding stoichiometry are obtained. A comparison of the measured and theoretical EDTA complexing capacity can be made.

Figure 15 shows the  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  titration data for titrations of  $6.25 \mu\text{M}$  EDTA at pH 6. Linear regression analysis of the titration data gives a  $\text{Cd}^{2+}$  complexing capacity of  $6.4 \mu\text{M}$  and a  $\text{Cu}^{2+}$  complexing capacity of  $6.4 \mu\text{M}$ , both values agreeing with the theoretical 1:1 metal ion/ligand stoichiometry. With a potent metal binding ligand such as EDTA, the dialysis titration technique provided an experimental result that was only in 2.4% deviation from theoretical cadmium(II) and copper(II) complexing capacities.

SFA titrations. SFA was used in the next series of titrations as a ligand that more closely resembles natural metal binding compounds than EDTA. This complicated mixture of compounds does not form metal ion complexes with well-defined stoichiometries. However, the extent of SFA complexation is known to be a function of the metal ion, pH, ligand concentration and ionic strength, based upon previous

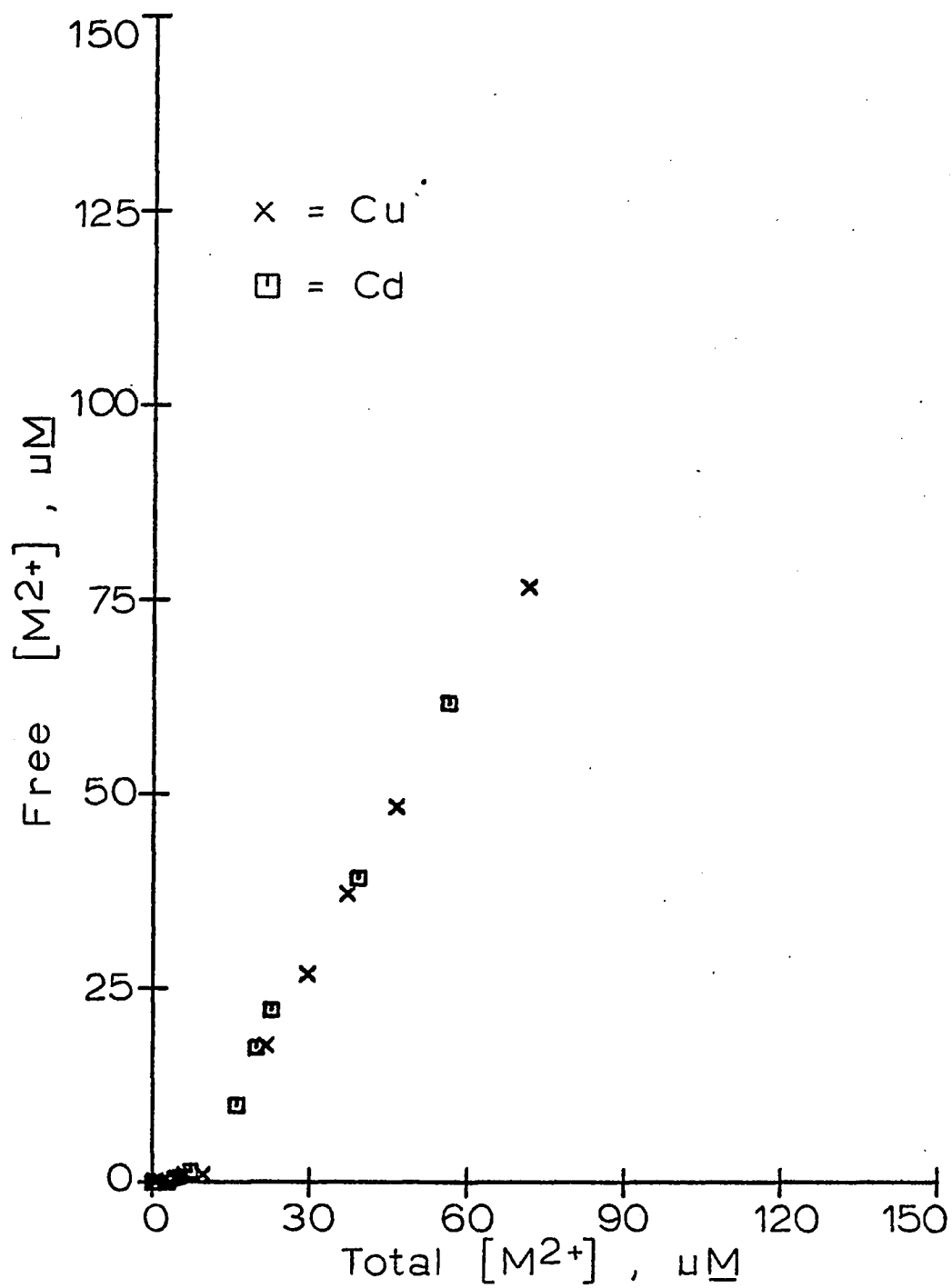


Figure 15.  $Cu^{2+}$  and  $Cd^{2+}$  Dialysis Titrations of  $6.25 \mu M$  EDTA vs.  $0.001 M KNO_3$  at pH 6

metal binding studies (Saar, 1980; Saar & Weber, 1979; Bresnahan et al., 1978). On the other hand, SFA solutions should have greater PCL's than natural water samples with the same UV absorbance (color) because of the absence of competitive cations. In other words, SFA solutions have no  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  NCL to diminish their complexing capacity.

Copper(II) (Figure 16) and cadmium(II) (Figure 17) titrations of 15.5  $\mu\text{M}$  SFA were performed at pH 5, 6 and 7. The copper(II) titrations were performed in triplicate at each pH, so Figure 16 is a plot of nine titrations. Figures 16 and 17 show differences between the titration behavior of the two metal ions. The different complexing capacities and slopes of copper(II) and cadmium(II) titrations, which are listed in Table 11, are graphed in Figures 18 and 19.

In theory, well past the metal ion complexing capacity, the titration curve slope of free vs. total metal ion should be one. In this region of the titration curve, metal ion complexation ability is exhausted so all metal ion additions should cause an equal increase in free metal ion. This behavior is seen in the EDTA complexing capacity titrations (Figure 15) and in  $\text{KNO}_3$  vs.  $\text{KNO}_3$  blank titrations, which will be described later (Table 11, slopes in sections A and C). The  $\text{Cd}^{2+}$ -SFA titration curves also have slopes of one beyond the inflection point (Table 11, section B). A striking difference between the  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  titration curves compared in Figures 18 and 19 is that only the pH 5  $\text{Cu}^{2+}$  titration curve has an upper branch slope of one.

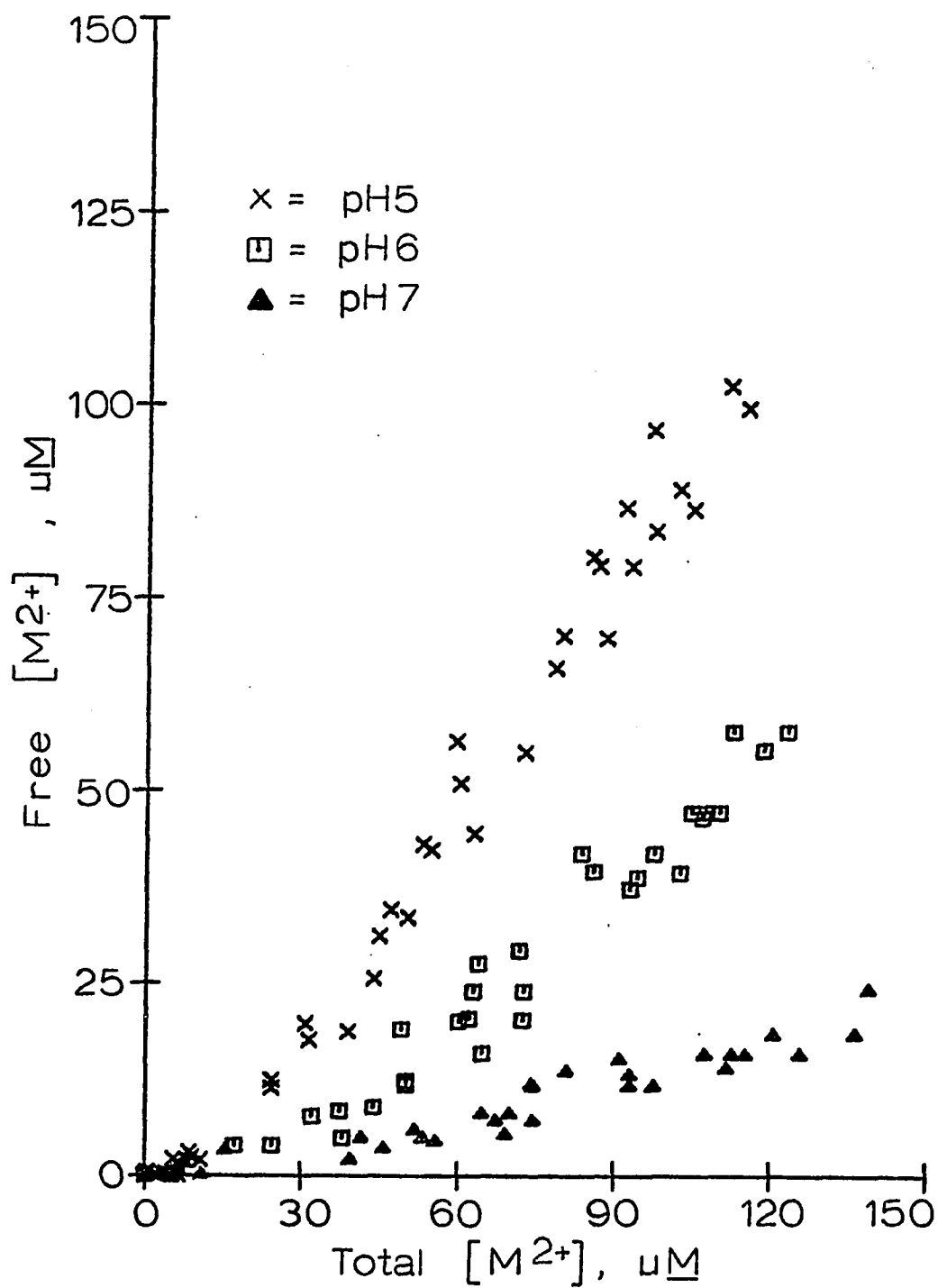


Figure 16.  $Cu^{2+}$  Dialysis Titrations of  $15.5 \mu M$  SFA vs.  $0.001 M$   $KNO_3$  at pH 5, 6 and 7

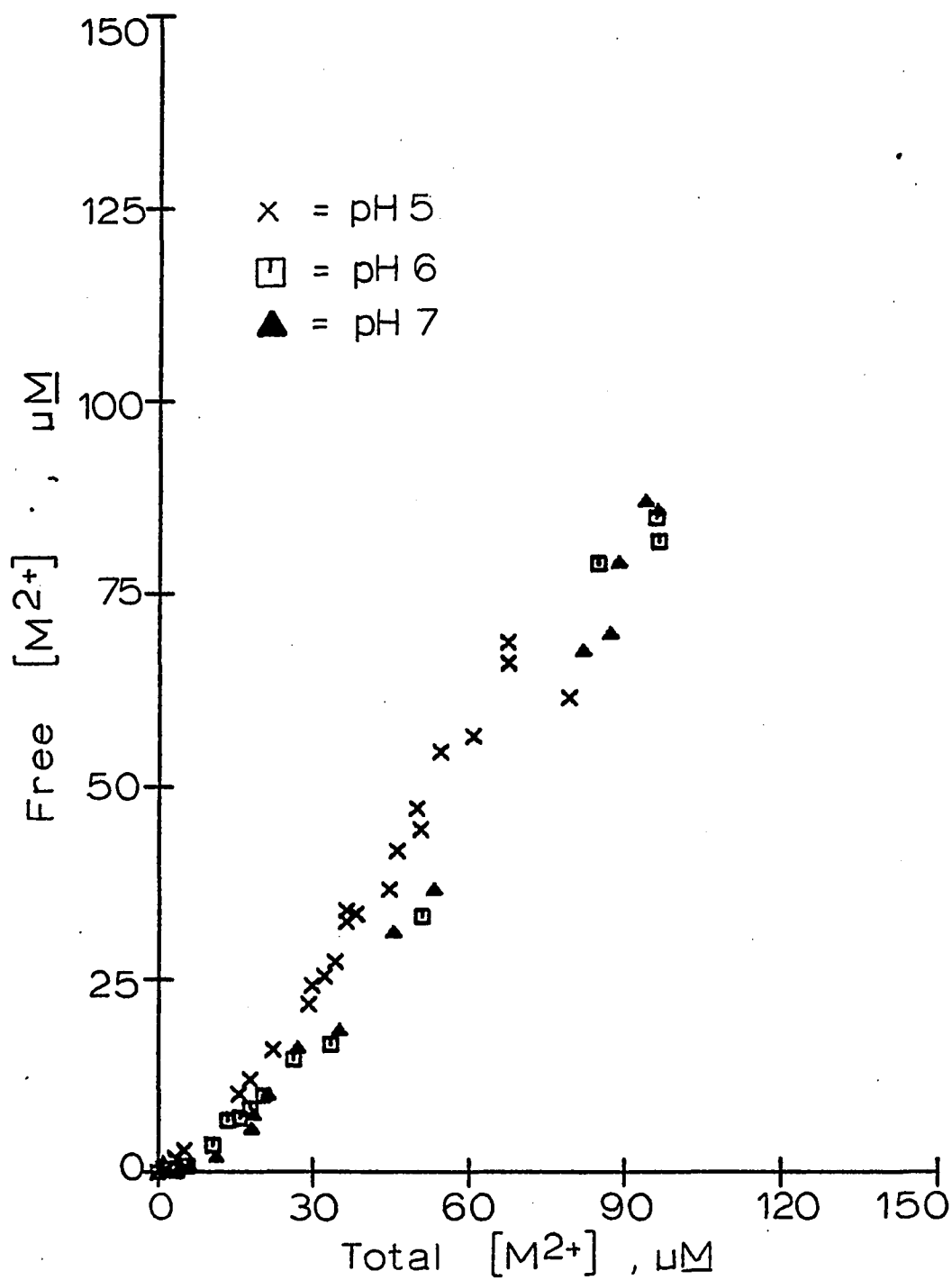


Figure 17.  $Cd^{2+}$  Dialysis Titrations of  $15.5 \mu M$  SFA vs.  $0.001 M$   $KNO_3$  at pH 5, 6 and 7

Table 11. Summary of All Dialysis/AA Complexing Capacity Measurements

<u>Titrated Solution</u>	<u>Titrant</u>	<u>Complexing Capacity (<math>\mu\text{M}</math>)<sup>d</sup></u>	<u>Regression Slope</u>	<u>Date</u>
A. 6.25 $\mu\text{M}$ EDTA <sup>c</sup> , pH 6				
	Cu	6.4	1.19	10/79
	Cd	6.4	1.23	10/79
B. 15.5 $\mu\text{M}$ SFA <sup>c</sup>				
pH 5	Cu	19.2 <sup>a</sup>	1.04	4/80
		16.4	1.12	3/80
		13.1	1.09	3/80
pH 6	Cu	21.8 <sup>a</sup>	0.56	4/80
		32.2 <sup>a</sup>	0.66	4/80
		18.4	0.50	5/80
pH 7	Cu	31.4 <sup>a</sup>	0.20	4/80
		15.1 <sup>a</sup>	0.15	4/80
		39.5	0.31	5/80
pH 5	Cd	8.0	1.10	3/80
pH 6	Cd	15.7	1.06	3/80
pH 7	Cd	19.3	1.11	3/80
C. 0.001 M KNO <sub>3</sub>				
pH 5	Cu	0.5	0.94	9/79
pH 6	Cu	-0.5	1.00	2/80
pH 6	Cd	-1.9	0.95	2/80
pH 7	Cu	1.0	0.96	2/80
pH 7	Cd	0.0	0.96	2/80
D. Natural Water Samples				10-12/79
Portsmouth	Cu	8.6	1.05	
Reservoir	Cd	-1.4	0.95	
Drew Pond	Cu	11.9	0.89	
	Cd	9.7	1.04	
Swamp	Cu	15.1	1.15	
	Cd	2.0	0.98	
Lamprey	Cu	10.7	0.92	
	Cd	0.4	0.97	
Durham	Cu	5.0	0.56	
	Cd	3.1	1.00	
Oyster River	Cu	1.1	0.61	
	Cd	n.d.	n.d.	
Exeter River	Cu	2.1 <sup>b</sup>	0.67	
	Cd	4.3	1.03	

<sup>a</sup>Data used in the paired t-test method comparison.

<sup>b</sup>Calculated from few data near the endpoint.

<sup>c</sup>SFA is soil-derived fulvic acid, EDTA is ethylenedinitri-lotetraacetic acid.

<sup>d</sup>This is potential complexing level (PCL) for natural water samples.

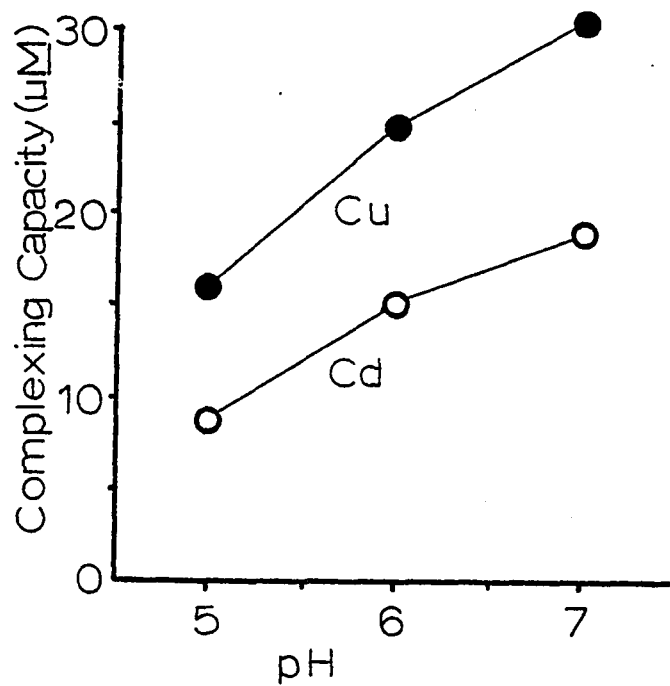


Figure 18. Average  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  Complexing Capacities from the Dialysis Titrations of  $15.5 \mu\text{M}$  SFA at pH 5, 6 and 7



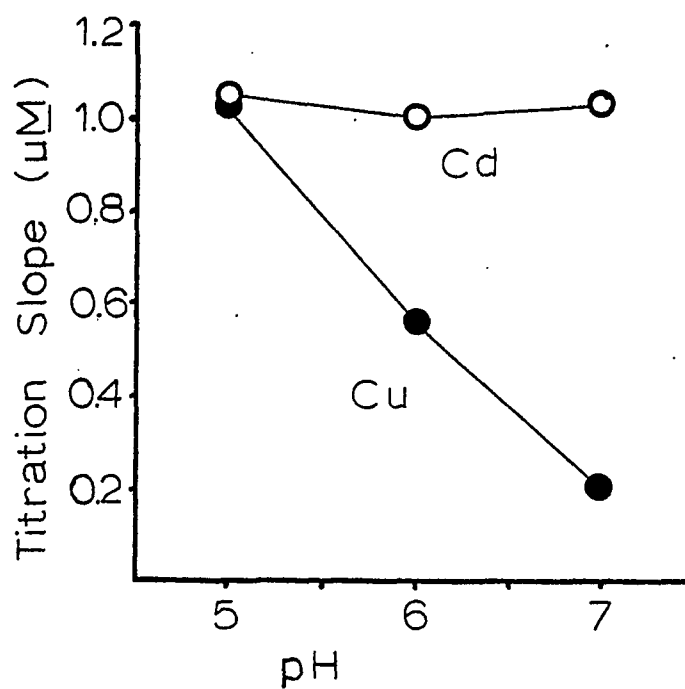
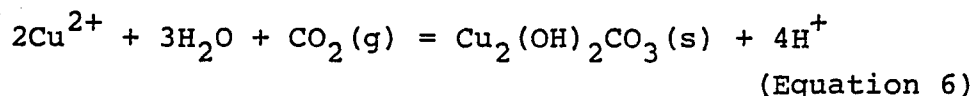
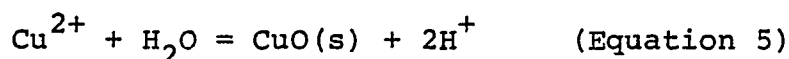


Figure 19. Average  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  Slopes from the Dialysis Titrations of  $15.5 \mu\text{M}$  SFA at pH 5, 6 and 7

At pH 6 and 7,  $\text{Cu}^{2+}$  diffusate levels are lower than expected, suggesting the formation of some copper(II) species in the retentate solution that does not permeate the dialysis membrane. McCrady and Chapman (1979) attribute the titration curve slope after the endpoint to inorganic complexation of the metal ion. In equilibrium with air at pH 6 and 7,  $\text{Cu}^{2+}$  solubility is restricted (total copper(II) is ca. 100  $\mu\text{M}$ ) by the formation of tenorite ( $\text{CuO}$ ) or malachite ( $\text{Cu}_2(\text{OH})_2\text{CO}_3$ ) (Baes & Mesmer, 1976):



Indeed, in the titrations where the data indicated pronounced inorganic complexation, a green precipitate was found. In contrast,  $\text{Cd}^{2+}$  has no tendency to form insoluble hydroxo or carbonate species at the experimental cadmium (II) concentrations. No evidence of complexation beyond the organic matter endpoint was found in any cadmium(II) titration. The  $\text{Cd}^{2+}$  SFA titration slopes (Figure 19) remain about one at pH 5-7.

As expected from ISE titration results with SFA (Bresnahan et al., 1978; Saar & Weber, 1979),  $\text{Cu}^{2+}$  complexing capacities of SFA are greater than  $\text{Cd}^{2+}$  values at the same pH, and the complexing capacities of both ions increase with pH (Figure 18, Table 11, section B). The mean  $\text{Cu}^{2+}$  complexing capacities and their standard deviations are

16.2 ( $\pm$  3.1) for pH 5, 24.1 ( $\pm$  7.2) for pH 6 and 28.7 ( $\pm$  12.4) for pH 7. The mean  $\text{Cu}^{2+}$  titration slopes (Figure 19) and their standard deviations are 1.08 ( $\pm$  0.04) for pH 5, 0.57 ( $\pm$  0.08) for pH 6, and 0.22 ( $\pm$  0.08) for pH 7. Differences between the mean values and the reproducibility data indicate that titration curve slope might be a better diagnostic property of the pH dependence of copper(II) complexing capacity than the intercept, particularly for low concentrations of ligand or for titrations of weak ligand systems.

The titration reproducibility problems are mostly caused by pH drift in the 48 hours between titrant additions. This problem is greatest at pH 7 where carbonate buffering acts to lower the solution pH to about 5.5. There are other factors, however, that might contribute to titration irreproducibility. For instance, the SFA may be degrading from oxidation or polymerizing during the titration period to different extents in each solution, especially at pH 7. Varying amounts of microorganism growth on the dialysis membranes, which inhibit metal ion diffusion, may also be occurring in the dialysis solutions. Finally, inhomogeneity in the dialysis membrane porosities would cause different SFA retentions to occur in the dialysis cells, thereby altering the measured complexing capacities and titration slopes.

$\text{KNO}_3$  control titrations. As experimental controls, a series of cadmium(II) and copper(II) dialysis titrations of

0.001 M  $\text{KNO}_3$  were performed at pH 5, 6 and 7. The titration results are found in Table 11 and graphed in Figure 20. These dialysis titrations were performed to identify titration effects that are not due to SFA.

The  $\text{KNO}_3$  dialyses in Figure 20 have slopes of about one and intercepts of essentially zero, indicating zero complexing capacities. One titration, the copper(II) titration at pH 7, is greatly distorted by inorganic copper(II) species formation. The slope flattens and there is a great increase in data scatter for copper additions beyond about 25  $\mu\text{M}$ . In this titration, a green precipitate could be seen after the dialysis cell solution was allowed to stand unstirred for a short time. The observed production of an insoluble inorganic species for  $\text{Cu}^{2+}$  concentrations greater than 25  $\mu\text{M}$  at pH 7 is a limitation to any copper(II) complexing capacity titration. No such precipitation effect was seen in any other of the  $\text{KNO}_3$  blank titrations.

#### Comparison of Dialysis and ISE Titration Methods

Prior fulvic acid complexing capacity determinations and the permeation properties of the dialysis membranes discussed earlier suggest that fulvic acid complexing capacity results are method dependent (Buffle *et al.*, 1978; Guy & Chakrabarti, 1976b; Florence & Batley, 1977). In this study dialysis/AA measurements are directly compared to the ISE results of five SFA titrations to determine if these methods yield the same titration results. These comparisons

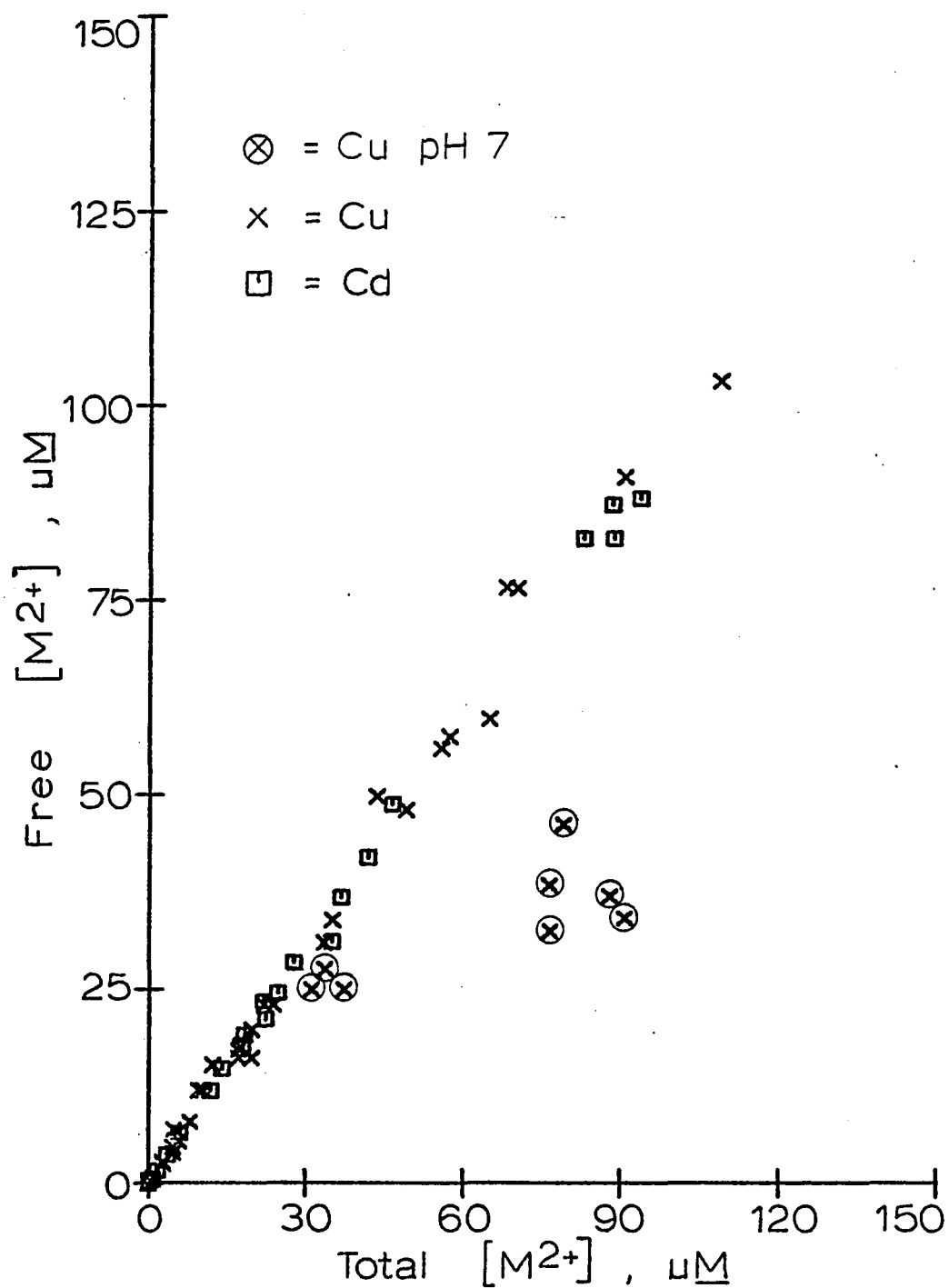


Figure 20.  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  Dialysis Titrations of 0.001  $\underline{\text{M}}$   $\text{KNO}_3$  vs. 0.001  $\underline{\text{M}}$   $\text{KNO}_3$  at pH 5, 6 and 7

are achieved by conducting a paired t-test where each SFA solution is titrated by both methods.

A comparison of dialysis/AA measurements and ISE potentiometric measurements is appropriate since copper(II) complexing capacity is most often determined by ISE potentiometry. Table 12 has the copper(II) complexing capacities of 15.5  $\mu\text{M}$  SFA determined at pH 5, 6 and 7 by dialysis/AA and ISE potentiometry. Following the procedure described earlier, the pairs of complexing capacity measurements were compared. A statistically significant difference between the pairs in Table 12 means that dialysis/AA and ISE potentiometric titrations of identical solutions do not yield the same result. Equation 3 and the data in Table 12 produce a calculated t (1.17) smaller than the tabular t for 4 degrees of freedom at the 95% confidence level (2.78). The t-test conclusion therefore is that the hypothesis of no difference between dialysis/AA and ISE potentiometry results cannot be rejected. Otherwise stated, the statistical test was unable to distinguish any significant differences between the average results in light of the large experimental variance.

#### Dialysis Titrations of Natural Water Samples

After being used to measure EDTA and SFA complexing capacities, the dialysis/AA titration method was applied to seven southeast New Hampshire freshwater samples. These samples are a range of freshwater types, including pond

Table 12. Paired t-Test Comparison of Dialysis/AA and ISE  
Titrations of 15.5  $\mu$ M SFA Solutions

<u>Sample</u>	<u>Dialysis Complexing Capacity</u>	<u>I.S.E. Complexing Capacity<sup>a</sup></u>
pH 7	15.1	43.6
pH 7	31.4	43.9
pH 6	32.2	26.1
pH 6	21.8	26.8
pH 5	19.2	22.7

<sup>a</sup>Average of three titrations

water, swamp water, and drinking water sources like rivers and reservoirs. Samples were taken from the Durham reservoir, Portsmouth reservoir, Lamprey River, Exeter River, Oyster River, Drew Pond and a swampy area in Barrington, N.H. (Figure 8). All the river samples were taken near water treatment plants.

Water sample characteristics. Several chemical properties of the freshwater samples were measured using established methods (Standard Methods for the Examination of Water and Wastewater, 1975) to provide a context for the measured metal ion binding abilities of each natural water sample. Correlations between these characteristic properties are sought statistically.

Each natural water sample was examined for pH, alkalinity, hardness, conductance, dissolved organic carbon content (DOC), color, dissolved iron, copper and cadmium levels, and  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  complexing capacities. Alkalinity and pH are considered characteristic of the bicarbonate, carbonate and hydroxide ion content of the sample (Stumm & Morgan, 1970), although any fulvic and humic acids present will also contribute to these properties. Alkalinity expressed as mg/L  $\text{CaCO}_3$  is actually the proton binding capacity of the sample, just as complexing capacity measures the metal ion binding ability. The alkaline earth ions most often associated with the alkalinity anions, like  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , are determined by the hardness titrations and are expressed as mg/L  $\text{CaCO}_3$ . Hardness ions in fact are all the



cations in solution that contribute to the EDTA titration endpoint, although the geologically available ions like  $Mg^{2+}$  and  $Ca^{2+}$  usually predominate. Conductance (mhos/cm) is a measure of the ionic strength of the samples. The organic matter concentrations of the water samples is determined by DOC and color measurements. A measurement of all the oxidizable organic matter in solution was obtained from DOC determinations. Color, on the other hand, is a measure only of the compounds contributing to the UV absorbance of the samples.

Table 13 has all the properties of the seven filtered natural water samples. Since they were all taken at the end of a month-long rainy period, the river samples have high color, pH, alkalinity and conductance (Giesy et al., 1978). Soil run-off and groundwater upwelling mechanisms are responsible for increased organic matter and suspended matter in river and reservoir water after heavy rain periods (Reuter & Perdue, 1977; Jackson, 1975).

Copper(II) complexing capacities (Figures 21-27), ranging from 2 to 12  $\mu M$ , are larger than the corresponding cadmium(II) complexing capacities, which range from about 0 to 10  $\mu M$ . Comparisons of all the water samples can be made except for the Oyster River and Exeter River samples. No comparison can be made between the copper(II) and cadmium(II) complexing capacities in the Oyster River sample because the cadmium(II) titration failed when microorganisms attacked the dialysis membrane (Figure 21). Copper(II)

Table 13. Natural Water Sample Characteristics

<u>Water Characteristic</u>	<u>Portsmouth Reservoir</u>	<u>Lamprey River</u>	<u>Oyster River</u>	<u>Exeter River</u>	<u>Durham Reservoir</u>	<u>Drew Pond</u>	<u>Barrington Swamp</u>
pH	6.3	6.6	7.3	7.4	7.4	6.4	5.7
DOC (mg/L as KHP)	6.8	11.8	11.4	12.5	7.7	12.0	11.8
Hardness (mg/L as CaCO <sub>3</sub> )	25	22	30	31	23	7.8	5.3
Color (mg/L as SFA)	12.8	19.6	16.3	11.0	19.0	16.5	17.0
Alkalinity (mg/L as CaCO <sub>3</sub> )	7.4	20	41	43.4	30.7	4.2	1.7
Conductance (µmhos/cm)	55.2	81.3	115	140	118	65.0	54.8
[Fe] µM	5.30	6.39	3.99	6.75	2.26	6.00	7.23
[Cu] µM	0.044	0.031	0.047	0.015	0.047	0.016	0.011
[Cd] µM	0.020	0.007	0.010	0.036	0.003	0.002	0.00
Cu PCL µM	8.6	10.7	1.1	2.1	5.0	11.9	15.1
Cd PCL µM	-1.4	0.4	n.d.	4.3	3.1	9.7	2.0
Cu Slope	1.0	0.92	0.61	0.67	0.56	0.89	1.15
Cd Slope	0.95	0.97	n.d.	1.03	1.00	1.04	0.98

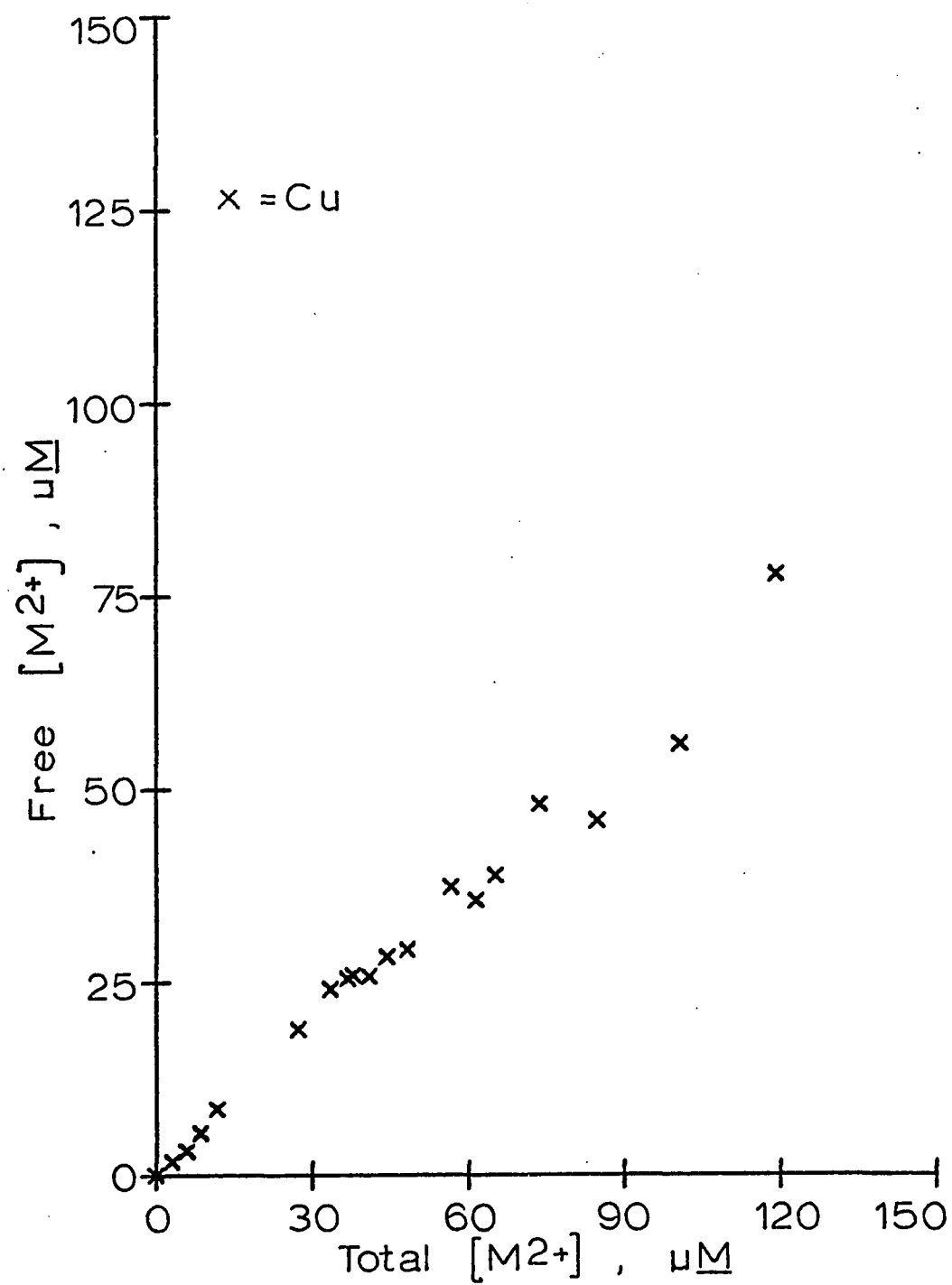


Figure 21.  $Cu^{2+}$  Dialysis Titration of Oyster River Water

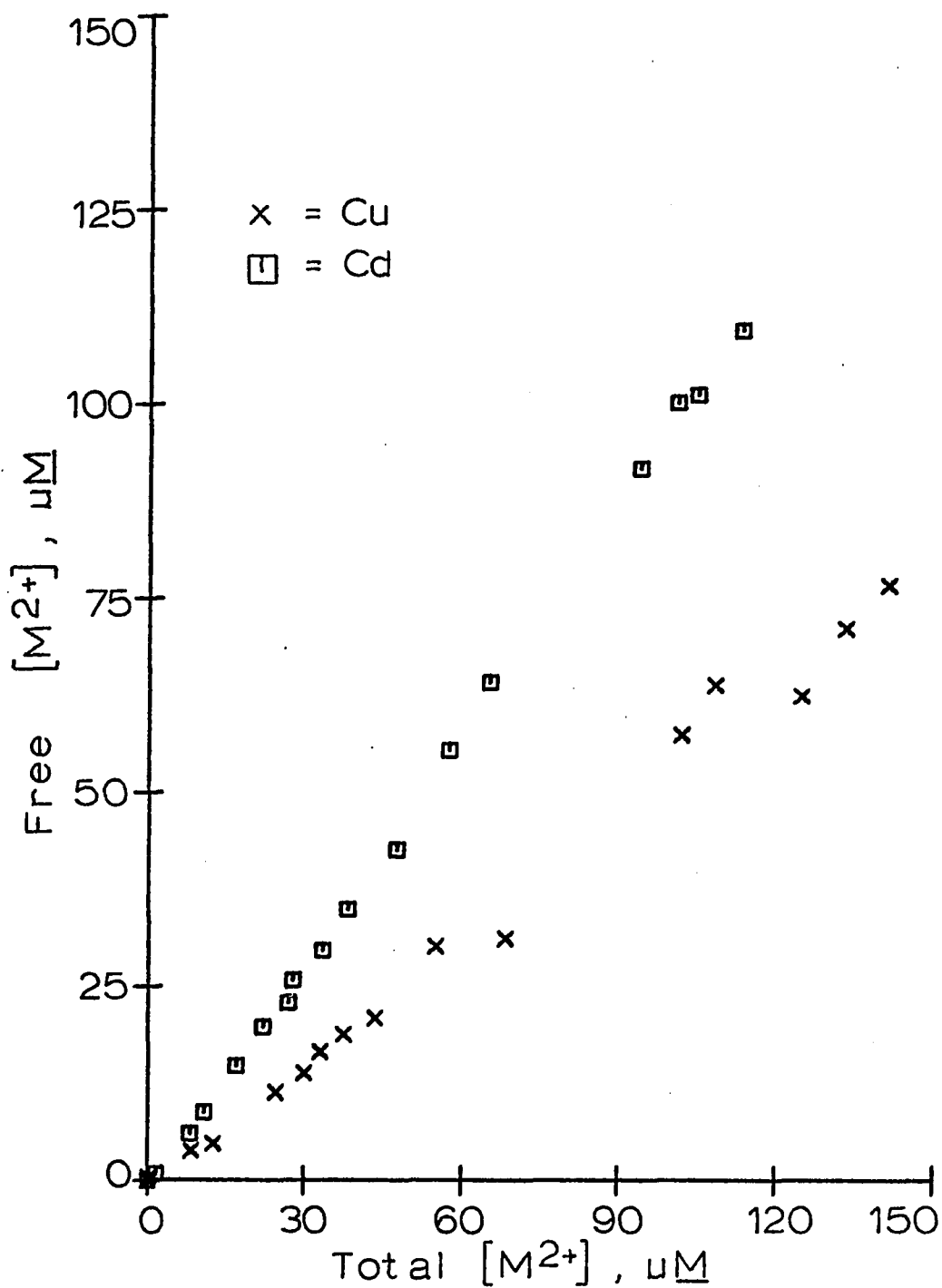


Figure 22.  $Cu^{2+}$  and  $Cd^{2+}$  Dialysis Titrations of Durham Reservoir Water

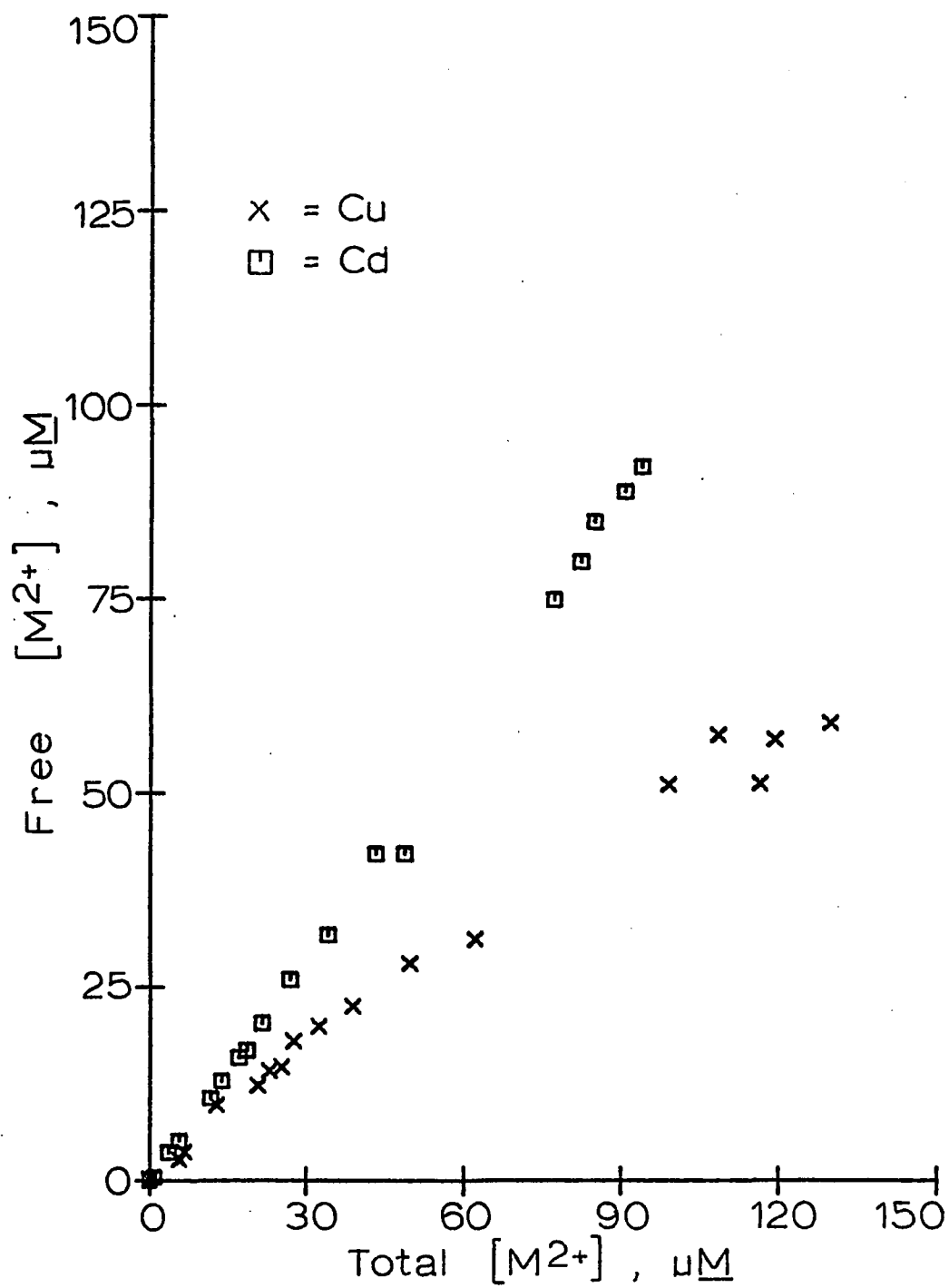


Figure 23.  $Cu^{2+}$  and  $Cd^{2+}$  Dialysis Titrations of Exeter River Water

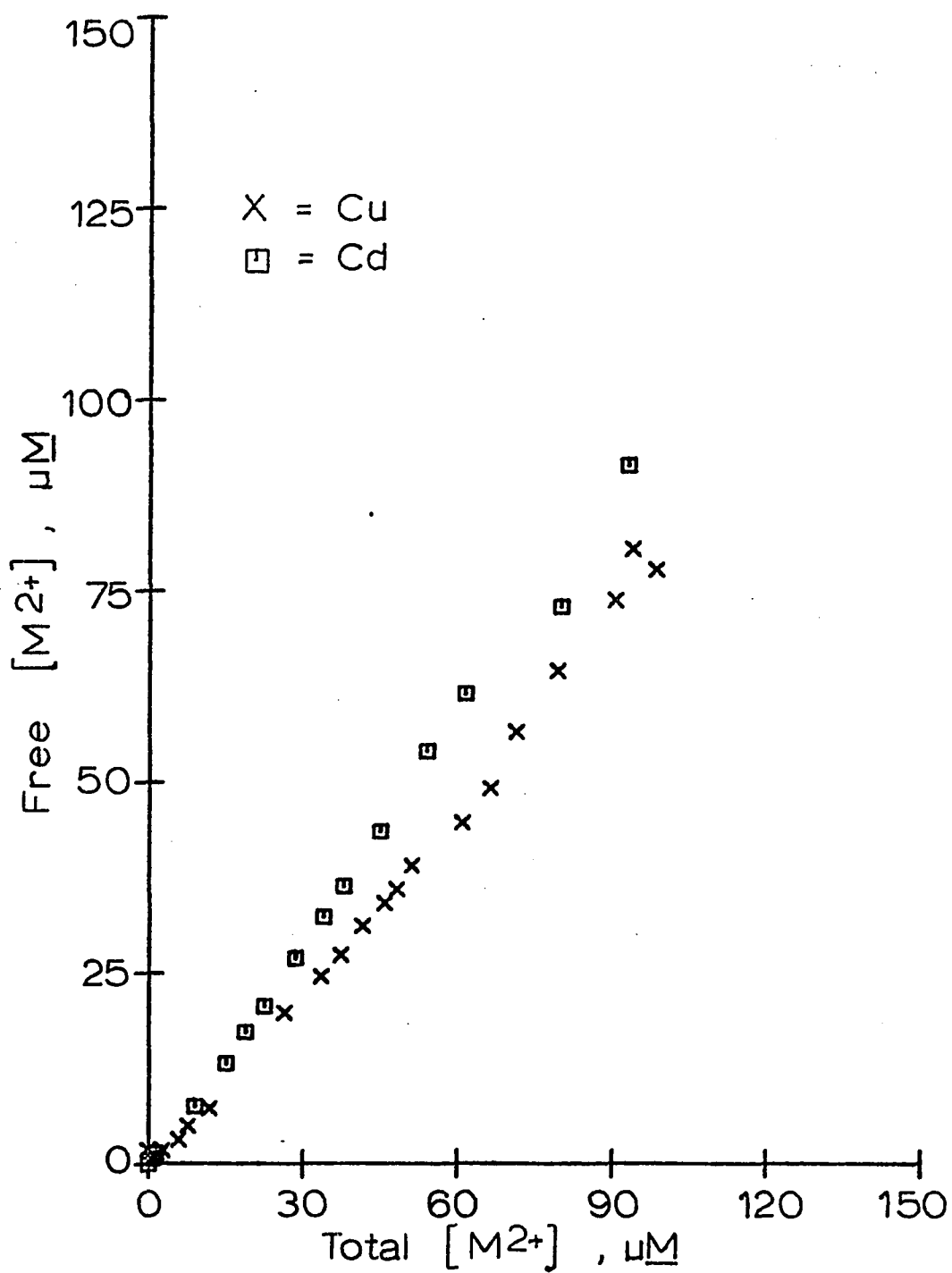


Figure 24.  $Cu^{2+}$  and  $Cd^{2+}$  Dialysis Titrations of Lamprey River Water

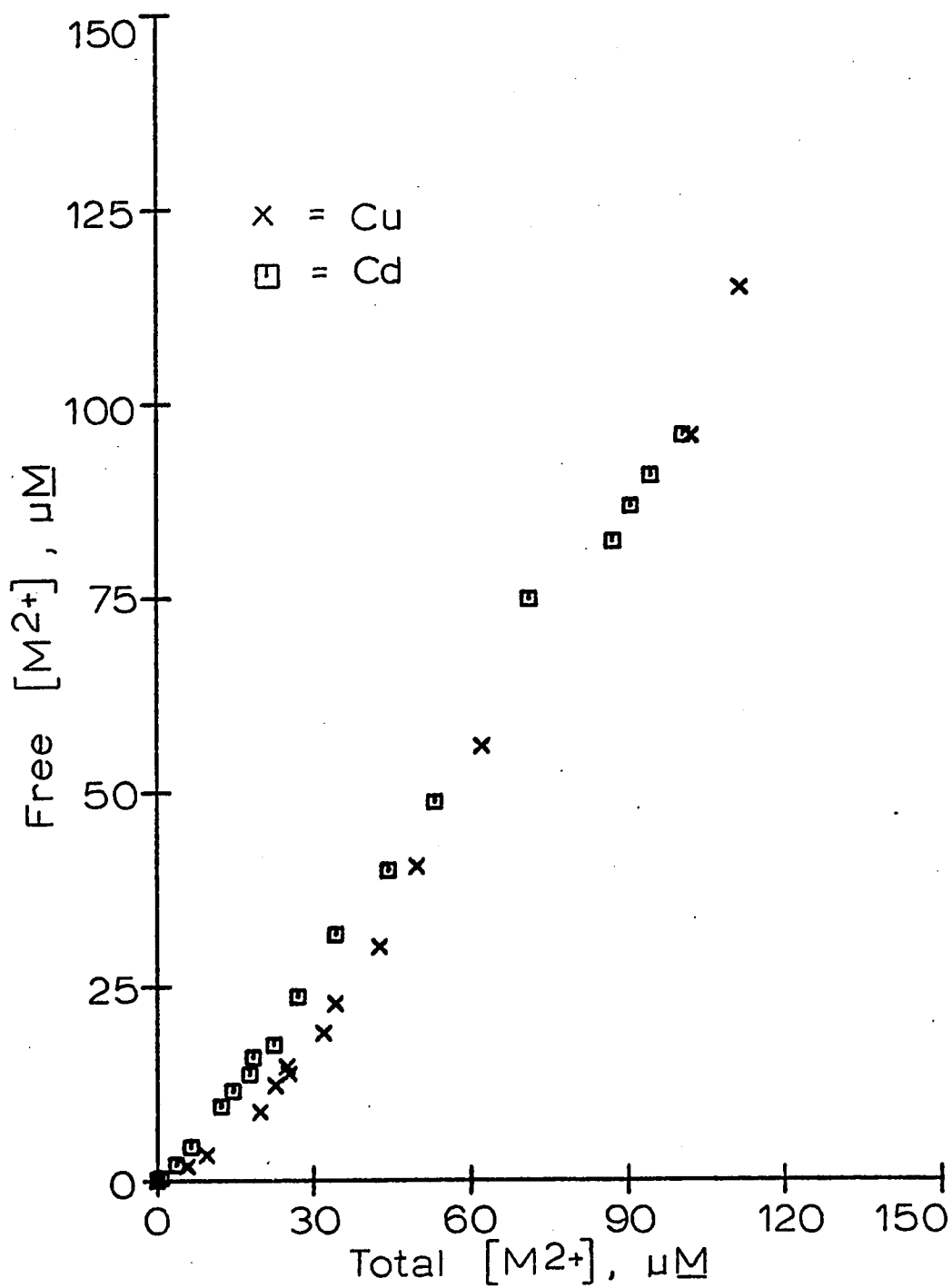


Figure 25.  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  Dialysis Titrations of Barrington Swamp Water

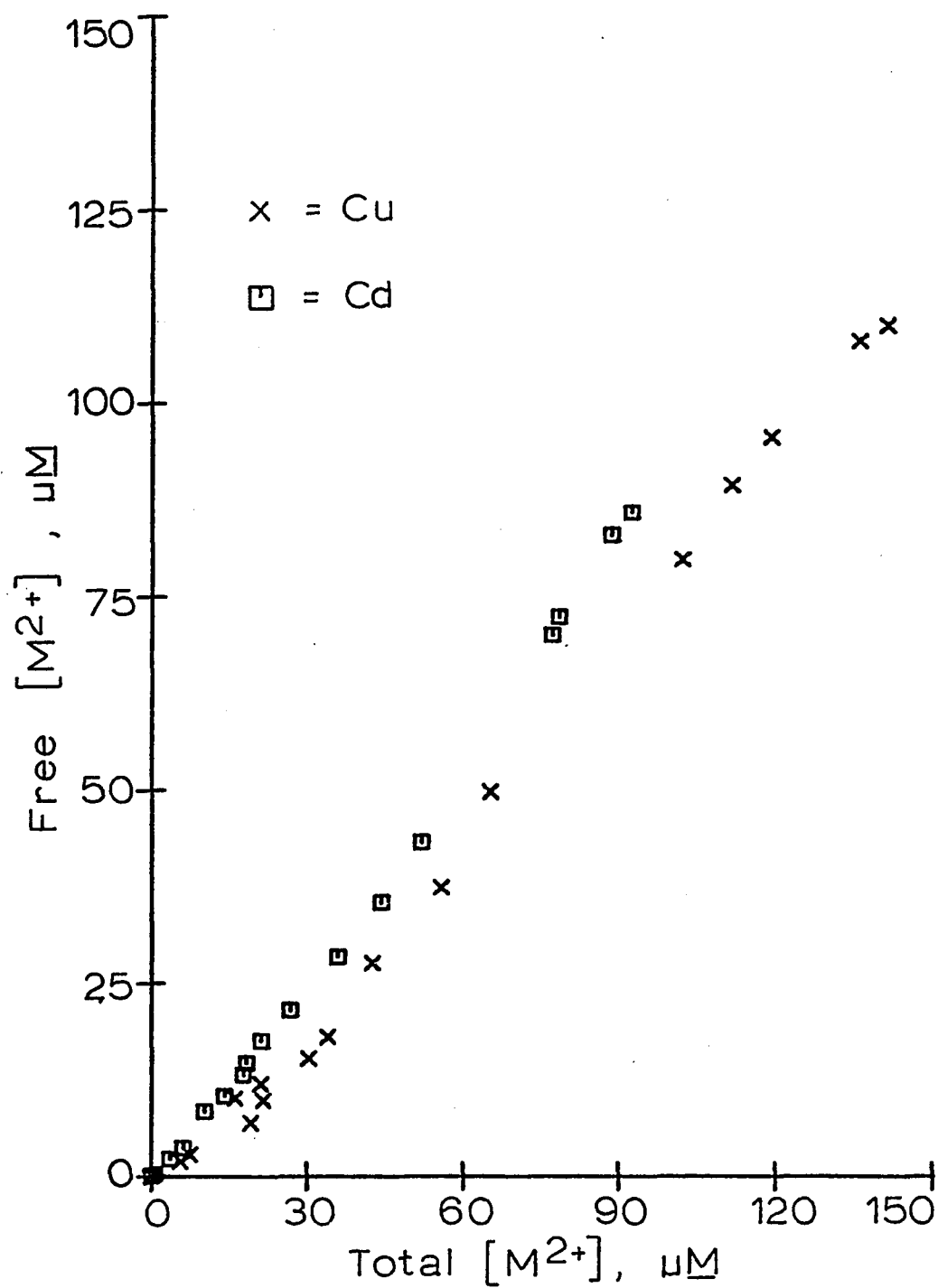


Figure 26.  $Cu^{2+}$  and  $Cd^{2+}$  Dialysis Titrations of Drew Pond Water



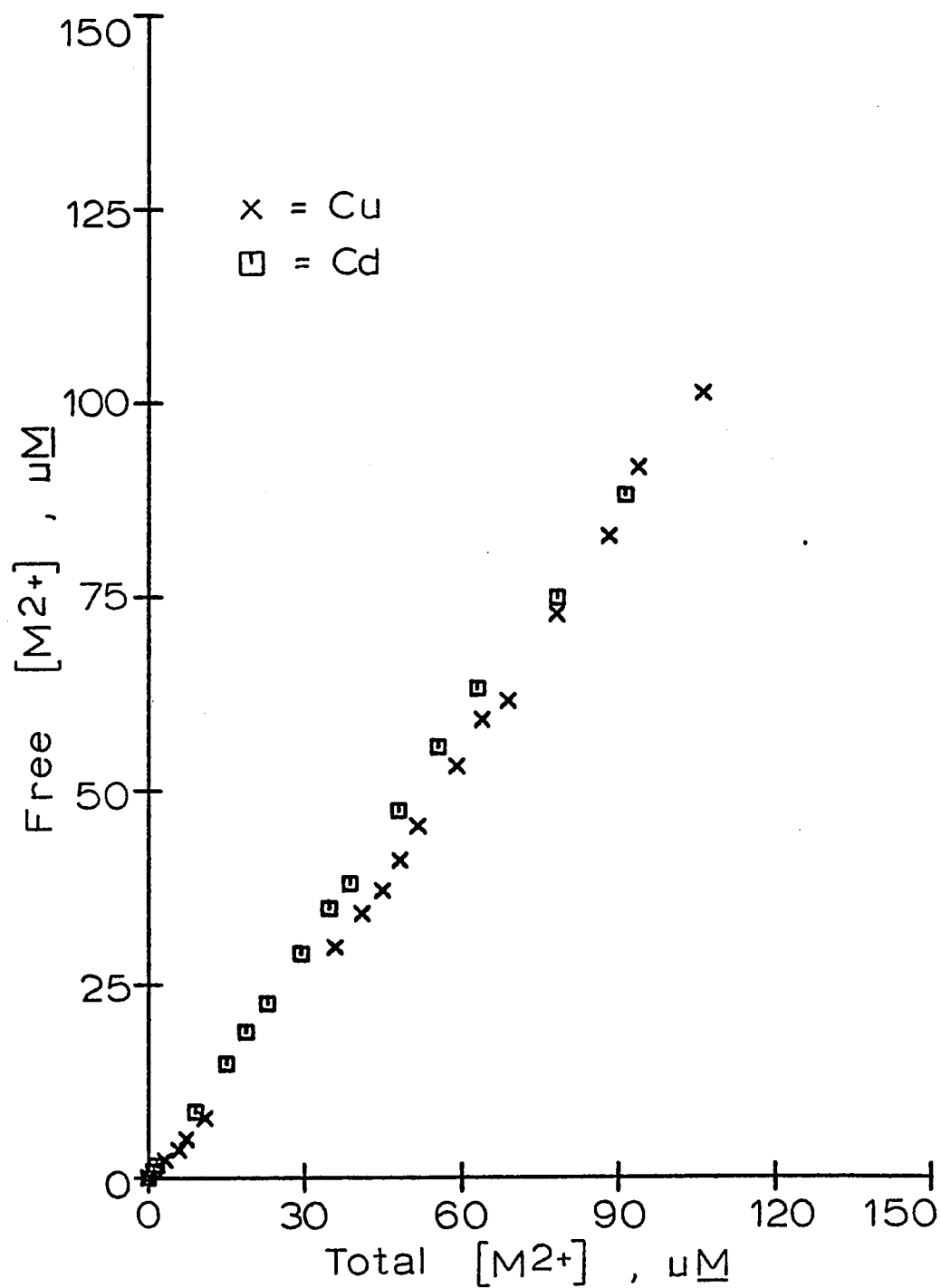


Figure 27.  $Cu^{2+}$  and  $Cd^{2+}$  Dialysis Titrations of Portsmouth Reservoir Water

apparently inhibited this microbial activity since no evidence of these organisms could be seen in the copper(II) titration solution or in the titration data (Figure 21). The Oyster River sample is the only one taken that exhibited this type of analytical interference, but obviously any water sample with biota is susceptible to adverse effects using the dialysis/AA measurement method. The difficulty is aggravated over long titration periods because biological growth conditions are optimized. First, the medium is provided with nutrients in the form of the cellulose membrane, then an incubation period is provided while the titration progresses. In the failed Oyster River sample titration, spots of growth could be seen and the titration data reflected inhibited  $\text{Cd}^{2+}$  diffusion suddenly after two weeks of the titration.

The Exeter River copper(II) titration (Figure 23) was short-lived due to the solubility limit of some copper(II) species. Soon after the titration began, a green precipitate appeared and uncomplexed copper levels did not increase with subsequent additions of metal ion. The copper(II) complexing capacity of the Exeter River reported in Table 11 is calculated from the few data prior to the interference and, as a result, is of dubious validity. This copper(II) precipitation interference, seen in the pH 7  $\text{KNO}_3$  blank titrations (Figure 20) and the  $\text{Cu}^{2+}$ -SFA titrations (Figure 16) is probably an inorganic species. The Exeter River has the highest pH of the samples taken, and the highest alkalinity

and conductance, all of which would cooperate to lower  $\text{Cu}^{2+}$  solubility through the formation of hydroxide ion, carbonate ion or mixed inorganic copper(II) precipitates.

Table 11 also has the complexing capacity titration curve slopes. The slopes are part of the list of natural water sample properties, and are included in the correlation study so that a comparison can be made with the results of McCrady and Chapman (1979), who indicated that the upper titration curve branch slope is a function of pH and alkalinity. In fact, these authors conclude that generally  $\text{Cu}^{2+}$  complexing capacity is a function of the organic matter content of the sample, while  $\text{Cd}^{2+}$  capacities are dominated by the inorganic constituents.

One other result of the dialysis titrations was measured. At the end of the Exeter River, Drew Pond, Swamp and Durham reservoir titrations, the color and fluorescence of all diffusate and retentate solutions were determined, just as they were in the SFA dialyses. Table 14 lists the diffusate to retentate ratios of both color and fluorescence values for the four environmental water samples. The color diffusate to retentate ratio ( $R_c$ ) measures the relative concentrations of UV absorbing organic matter that has passed the dialysis membrane. Similarly, the fluorescence ratio ( $R_f$ ) measures the permeation of fluorescing compounds. Whether the natural organic matter fluorescence can be quenched by  $\text{Cu}^{2+}$  complexation like SFA is not known.

In the  $\text{Cu}^{2+}$  titrations, the  $R_c$  values range from 0.42

Table 14. Color and Fluorescence Intensities of the Exeter River, Drew Pond, Barrington Swamp and Durham Reservoir Samples at the End of Their Dialysis Titrations

<u>Water Sample</u>	<u>Titrant</u>	<u>Diffusate/Retentate</u>	
		<u>Color (R<sub>c</sub>)</u>	<u>Fluorescence (R<sub>f</sub>)</u>
Drew Pond	Cu	0.62	0.86
	Cd	0.40	1.03
Durham Reservoir	Cu	0.56	1.12
	Cd	0.70	0.99
Exeter River	Cu	0.42	0.92
	Cd	1.00	0.98
Swamp	Cu	0.71	0.98
	Cd	0.43	1.06

to .71 with the Exeter River sample having the lowest ratio and the swamp water sample having the highest. Referring to the other properties of these samples, the Exeter River also has the highest pH, hardness, conductance and alkalinity, while the swamp water has the lowest (Table 13). The  $R_f$  values of the same solution indicate that the internal and external dialysis bag solutions have essentially the same concentration of fluorescing material.

In the  $Cd^{2+}$  titrations of the four natural samples, the  $R_c$  trend is opposite the  $Cu^{2+}$  titration trend (Table 14). Here the Exeter River  $R_c$  is the greatest and both the swamp water and Drew Pond samples have the lowest  $R_c$ . Once again though, the  $R_f$  values indicate that diffusate and retentate levels of fluorescing material are equal.

It is not clear how the natural water organic matter color and fluorescence relate to the metal binding ability of the samples, except to note from the natural water property correlation study that will be discussed, that color and complexing capacity have no relationship. The color and fluorescence permeation described by the  $R_c$  and  $R_f$  ratios in Table 14 show that some natural organic compounds are more successful in diffusing through the 1000 m.w.c.o. dialysis membranes than SFA solutions with the same color and pH. The natural samples then, contain more small molecules that absorb 260 nm light than comparable SFA solutions and fluoresce at 435 nm more than SFA. SFA solutions are different from the natural samples in metal ion complexing

capability as well (Table 11). An important difference between SFA solutions and these hard freshwater samples which would account for metal ion binding differences is the SFA solutions do not have relatively high concentrations of cations to compete with the titrant metal ion for the available complexing capacity, as the natural water samples do.

Correlation of natural water sample properties. Correlation coefficients were calculated for all of the properties (Table 13) of the Portsmouth reservoir, Lamprey River, Exeter River, Durham reservoir, Drew Pond and Swamp water samples (the Oyster River sample properties were not included since its  $\text{Cd}^{2+}$  PCL was not determined). From these correlations, the existence of significant relationships between the sample properties can be seen. In particular, the trend of any characteristic with the dialysis titration results (slope and intercept) is sought.

The correlation coefficients are shown in Table 15, where underlined coefficients are correlations assigned significance with a 0.1 probability of error (there are 4 degrees of freedom,  $R = .729$ ). Keep in mind that while the underlined coefficients identify trends between sample properties, they do not imply cause and effect relationships. In fact, a change in one sample characteristic corresponding with a change in another property (which is all the correlation coefficient measures) can be purely coincidental, or may arise from a complicated series of indirect effects that completely frustrates the assignment of any relationship

Table 15. Correlations of the Natural Water Characteristics<sup>a</sup>

Characteristic	DOC	HARD.	COLOR	ALK.	COND.	[Fe]	Copper PCL	Cadmium PCL	Copper Slope	Cadmium Slope
pH	-.119	<u>.755</u>	-.144	<u>.925</u>	<u>.933</u>	-.529	<u>-.930</u>	.156	<u>-.967</u>	.640
DOC		-.316	0.004	.073	.122	<u>.745</u>	.226	.501	.094	.504
Hardness			-.435	<u>.800</u>	.685	-.272	<u>-.880</u>	-.407	-.569	.173
Color				-.250	-.221	-.338	.462	.013	-.009	-.250
Alkalinity					<u>.980</u>	-.259	<u>-.910</u>	-.001	<u>-.844</u>	.670
Conductance						-.296	<u>-.887</u>	.164	<u>-.897</u>	<u>.788</u>
[Fe]							.414	.045	.617	-.014
Copper PCL								.015	<u>.832</u>	-.555
Cadmium PCL									-.317	.669
Copper Slope										-.702

<sup>a</sup>Underlined correlation coefficients have 90% confidence level significance  
(d.f. = 4, R = .729)

between the correlated properties.

Two general conclusions can be made from the correlations in Table 15. First, the strong correlations are found exclusively between the inorganic aspects of the water samples, chiefly the positive trends between alkalinity and conductance, hardness and alkalinity, and the pH correlations with hardness, alkalinity and conductance. In general, pH trends seem to influence the other water qualities with the notable exceptions of color, DOC and the cadmium(II) titration parameters. The correlations of the inorganic water properties are not surprising considering their mutual dependence by definition.

Second, the organic matter content of the water samples is remarkably inconsistent in its trends with other water properties, as shown in the almost universal poor correlations of DOC and color. The positive correlation of DOC with iron content can be rationalized as indicative of the iron complexation of dissolved humic matter. An interesting observation is that color and DOC have essentially no correlation. Indeed, color shows no trends at all. While DOC and color were supposed to be indicators of humic content and binding capacity, their mutual independence confirms the SFA permeation study results which concluded that color is not a good indicator of humic matter binding capacity. The correlation results could also reflect the small range of colors between the water samples (11.0 to 19.6 mg/L as SFA). That is, the statistics were unable to detect any trends in



this small range of values.

The correlations originally of interest are those involving the dialysis titration parameters, slope and PCL (intercept). The  $\text{Cd}^{2+}$  titration results show only one trend: when the conductance increased, the slope increased. There was little spread between all the  $\text{Cd}^{2+}$  titration slopes, which would make trends difficult to discern, and  $\text{Cd}^{2+}$  PCL's were generally small (with the exception of Drew Pond), further obscuring trends.

On the other hand, the  $\text{Cu}^{2+}$  titration results varied more than the  $\text{Cd}^{2+}$  results, and some trends were identified. Copper(II) PCL's showed negative trends with pH, alkalinity, conductance and hardness, and copper(II) slopes correlated negatively with pH, alkalinity and conductance and positively with  $\text{Cu}^{2+}$  PCL's.

The strong negative correlations of  $\text{Cu}^{2+}$  PCL's with water properties measuring  $[\text{Ca}^{2+}]$ ,  $[\text{Mg}^{2+}]$ ,  $[\text{HCO}_3^-]$  and  $[\text{OH}^-]$ , and the further evidence of small measured PCL's in solutions with high color properties (Table 13) are indicative of large concentrations of cations in the water samples. Some of these samples were taken during a prolonged rainy period where extensive soil run-off contributes to the water sample constituency. Under these conditions, the hardness metals ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ),  $\text{Na}^+$ , and  $\text{K}^+$  are all loaded into the water systems, accompanied by typical anions like carbonate, bicarbonate and hydroxide. As a result, the samples exhibiting unusually high pH and alkalinity also have high

content of hardness ions and low PCL's. The competitive effect of the natural water hardness cations is apparently important enough to lower their  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  complexing capacity (Stumm & Morgan, 1970, p. 283).

The correlation study results indicate that inorganic constituents, like alkalinity and pH, influence the copper (II) and cadmium(II) binding capacity more than the organic parameters (DOC and color). Two recently published investigations, where  $\text{Cu}^{2+}$ -ISE titration binding capacities were correlated to other natural water sample properties, have similar conclusions about the relative importance of the organic matter in metal binding ability. McCrady and Chapman (1979) used natural river water, well water and artificially reconstituted water in their study and conclude that complexing agents other than simple inorganic species ( $\text{OH}^-$ ,  $\text{CO}_3^{2-}$ ) define the titration curve at low copper(II) levels, but the upper titration curve slope is dominated by pH and alkalinity. Their river samples, taken in the northwest U.S., had a pH range from 7.0 to 8.5, and high alkalinities (24 to 219 mg/L as  $\text{CaCO}_3$ ). Giesy et al. (1978), who sampled southern Maine and New Hampshire rivers and lakes, conclude that  $\text{Cu}^{2+}$  complexation was largely associated with organic matter, while  $\text{Cd}^{2+}$  binding was chiefly inorganic species controlled. Their samples had a pH range of 4.6 to 6.3, and low alkalinities (1 to 30 mg/L as  $\text{CaCO}_3$ ). The differences between the three studies is apparently due to the differences in the composition of the water samples.

In soft, non-alkaline, acidic, colored northeastern water systems, the dissolved organic matter has an appreciable metal binding influence (Giesy et al., 1978). The same water systems, sampled during an extended rainy period, exhibit different metal binding properties due to the soil run-off loading of the water, principally with inorganic compounds (this study). The metal binding chemistry of the low organic concentration, high pH and alkaline northwest waters, however, is principally dominated by inorganic species (McCrady & Chapman, 1979).

There are two principal limitations to the correlation results. The first consideration is that there are only six samples (4 degrees of freedom) from which the trends are identified. This is a small sample size to make any generalizations about water characteristic trends and it is not a large enough sample size to identify weak correlations. The insensitivity from using such a small sample size is eased to some extent by the dissimilarity of the samples. As the range of correlated water properties increases, the ability to detect relationships increases. I chose to sample the swamp and pond waters because of their dissimilarity to the river water samples. Finally, if there is a large variance in any of the sample property measurements, a correlation may be assigned significance where none exists or insignificance can be assumed when a trend indeed exists as a result of a bad measurement. Of all the water properties only the dialysis titrations were not performed in

replicate because of the sample quantity limitations, so the PCL's and titration slopes in the correlation analysis are particularly susceptible to this type of error.

### Conclusions

Since none of the analytical methods reported to measure natural water metal ion binding capacity are free of mitigating complications, dialysis/AA does seem to be a useful alternative method, especially for metal ions that cannot be studied by ISE titration. Even with the smallest porosity membranes available however, some of the organic matter in environmental samples undoubtedly permeates the dialysis membranes (based upon the SFA and natural water diffusion studies), which might cause a low estimate of the natural water potential complexing level. This difficulty is minimized by reducing the duration of the titration.

The methods comparison by a t-test found no discernable difference between ISE and dialysis/AA titration results in spite of the less than ideal retention properties of the dialysis membrane. The dialysis method reproducibility is about 4-12% for 15.5  $\mu\text{M}$  SFA complexing capacity depending on pH. The technique is much more sensitive to the metal binding ability of powerful ligands than it is to weak or un-specific coordinating systems such as SFA or natural water samples.

In the future, metal complex formation constants may be obtained from this method. Dialysis/AA may prove to be the

best technique applicable to the measurement of iron and aluminum PCL's in natural water systems. Both of these metals are analytically difficult to manipulate and determine, particularly when the sample matrix must be undisturbed. Refinements of the dialysis method, which should be examined, include performing the titrations in smaller batches with a series of dialyses, one for each data point, run for only 48 hours. This would minimize the titration period and overcome most of the undesirable effects caused by protracted titration times.

## CHAPTER 5

### CONCLUDING REMARKS AND SUGGESTIONS

The water treatment experiment, the filtration experiment and the complexing capacity studies all have conclusions and summaries at the end of their respective chapters, but some concluding remarks about trace metal ion analysis of environmental water samples in general can be made from all the work in this dissertation. These comments are collected in this chapter.

#### Measuring Natural Water Complexing Capacity

The dialysis/AA analysis technique evaluated in Chapter 4 has limitations in its ability to measure the metal ion binding ability of environmental water samples, as do all the other techniques that have been applied to this analysis. The greatest limitation of the dialysis method is that, to a certain extent, the desired separation of bound from free metal ion is not achieved. This failure to isolate dissolved compounds based upon their size is inevitable in natural water samples where there are dissolved species in a continuum of sizes from protons to suspended clay and sediment particles. The consequence: the dialysis titration method does not measure the potential complexing level of a freshwater sample. It measures the PCL of those compounds retained by the dialysis membrane, and

that is better than 90% of all the metal ion binding compounds (Chapter 4). One problem that is inherent to the dialysis titration method is that the dialysis membranes occasionally become sustenance or desirable residences for microorganisms, which interfere with the separation ability of the membranes.

All complexing capacity analysis methods are limited by the solution chemistry of the analyte ions. The solubility limit of copper(II) seen in the pH 7  $\text{KNO}_3$ , the pH 6 and 7 SFA, and the Exeter River titrations would be seen by any titration technique. Finding a titration endpoint becomes more difficult as the concentration of dissolved free metal ion between the PCL (titration endpoint) and the solubility limit of the metal ion gets smaller. This limitation is probably severe for easily hydrolyzed ions like  $\text{Fe}^{3+}$ .

The dialysis titration method is the least objectionable technique for measuring the greatest variety of metal ion complexing capacities.  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  complexing capacities are probably best done by ISE titration. There are, however, no good alternatives for many other ions. The dialysis titration technique is worth more study and can be improved.

Specifically I would suggest two areas of further work with dialysis titration measurements of solution complexing capacities. First, the method might be improved by running a series of single titration point dialyses (everything

from one dialysis solution to the next is the same except for increased metal ion concentration) for a short period rather than titrating one solution over a long period. Most of the problems I encountered with this dialysis technique, like organic matter permeation of the membrane and microorganism attack on the membrane, can be minimized by shortening the titration period. Unknown aging reactions in the titration solution, like oxidation or polymerization, could be minimized also. This modification of the dialysis titration method is already being investigated in Dr. J.H. Weber's research group.

My second suggestion is to incorporate dialysis titration with all the other available complexing capacity determination methods in a comprehensive strategy for the measurement of natural water complexing capacity. No technique is superior to all the others in all applications. The specific restrictions of the analyte ion or the water sample may make one technique more applicable than the others, and all methods may have some, but different, limitations. In this case, measurements by more than one method may be needed. To achieve this comprehensive strategy, a comparison of some of the more powerful methods is needed. In a study similar to the paired t-test of Chapter 4, the results of ASV, ISE ( $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ ), dialysis/AA and possibly biological complexing capacity titrations should be compared.



### Modelling Natural Water Systems

The soil fulvic acid solutions used in these studies are simplistic models of natural water systems. These solutions provide analyte metal ions an environment with little ligand binding competition from alkali, alkaline earth, and other metal ions naturally found in such relative abundance in river, lake and swamp waters. Because SFA is only the acid soluble fraction of soil organic matter, natural systems are more complicated solutions of organic compounds. Finally, SFA solutions cannot model effects of living organisms found in natural solutions.

SFA solutions do, however, resemble natural water samples in their analysis problems. There is no analytical procedure that specifically determines the concentration of dissolved humic compounds. Data in Chapter 4 show that diagnostic characteristics like metal ion binding capacity, DOC, and color of natural water samples do not correlate. The SFA titrations in that chapter showed different color, fluorescence and metal ion binding ability of the dialyzed organic matter compared to the dialysis retentate compounds. To improve SFA as a model of natural organic material, the relationship of SFA color, fluorescence and metal ion complexing capacity should be clarified.

Although dissolved species were deliberately isolated for investigation, more realistic model natural water studies are possible than the SFA studies done in Chapter 2 and 4. Clay particulates can be introduced to the model

solutions so that metal ion and dissolved organic matter associations with suspended particulates, which are important natural phenomena, can also be studied. Since there is no need to filter natural water samples prior to measuring their metal ion complexing capacities by dialysis/AA, metal ion sequestering by suspended matter and colloidal compounds in complexing capacity determinations can be distinguished from complexation by dissolved ligands if metal ion binding is measured in filtered and unfiltered sample. This experiment would provide more information on metal ion speciation in natural systems. Finally, mixed metal ion studies should be performed to measure competitive binding in natural water systems.

#### Analytical Methodology

The analysis of metal species in environmental samples has some formidable requirements. Trace ( $\mu\text{g}$  to  $\text{mg/L}$ ) metal measurements are needed, analyte speciation is often required, and the analysis must leave the natural equilibria undisturbed. While trace metal analysis techniques are usually available, the additional need to identify the chemical form(s) of the analyte metal with a benign analysis method is a severe restriction. Natural water chemical equilibria are very complicated and can easily be altered when samples are taken, when the samples are manipulated prior to analysis, and during the analysis itself.

To accomplish these difficult analyses, throughout this dissertation I have emphasized some experimental

principles necessary for the analysis of metal species in natural water samples. One of these principles, the subject of the filtration experiment in Chapter 3, is to take precautions against sample changes. While obtaining and preserving representative samples has often been considered (Batley & Gardiner, 1977; Beneš & Kopicčka, 1976; Beneš & Steinnes, 1975; Isaaq & Zielinski, 1974; King, Rodriguez & Wai, 1974; Robertson, 1968; and Struempler, 1973), sample integrity during and in preparation for analysis is often not emphasized. In general, contamination and/or analyte species losses can be decreased by considering vessel cleaning procedures and controlling the materials of all surfaces the sample contacts through the analysis. However, simply limiting sample manipulations is the best procedure to minimize sample alterations.

Another principle I emphasized in my experiments is the evaluation of all analytical technique limitations in their application to natural water measurements. River, lake and swamp water matrices may have constituents that interfere with some measurements, and unless these analysis interferences are identified they cannot be distinguished from the chemical properties of the sample which are being investigated. Two well-known tactics can be used to evaluate analytical effects; the use of experimental controls (blanks) and the analysis of model systems. Analytical control experiments provide background levels of the measured parameters which can be used as a context for evalua-

ting the results of the actual sample. In my experiments, background metal ion filtration losses were determined for the water treatment study (Chapter 2) and the filter apparatus evaluation (Chapter 3), and electrolyte solutions were titrated by the dialysis complexing capacity measurement technique (Chapter 4). In all cases background effects were found.

To illustrate the benefits of analyzing model systems, SFA and EDTA solutions were used to evaluate the dialysis titration complexing capacity technique. From these model studies, ideal dialysis behavior and the effects of inorganic copper(II) processes were demonstrated before natural water samples were analyzed. As a result, the natural sample complexing capacity results can be understood in light of the analysis limitations.

The final experimental principle I have emphasized in my research is the use of statistical experimental design. This tool is well suited to aide investigations of poorly understood, analytically difficult, complicated systems like environmental waters. With the large amount of data that can accumulate from background experiments and the definition of natural water processes, experimental design can be used to gather the data efficiently (that is, only the information needed), and in such a way that the data can be statistically interpreted. The statistical analysis can identify trends, differences, or effects that are not easily seen from the collective data. As an example, the

influence of fulvic acid on metal ion removal in the water treatment experiment would have been misunderstood but for the ability of ANOVA to isolate the effects of variable interactions. Overall, the statistical approach minimizes subjective interpretation of results, makes data collection more efficient, and distinguishes random error from the data trends sought in the experiments. These are great advantages in a challenging and important field of research, the study of natural water chemistry.

APPENDIX

## CALCULATION OF THE VARIABLE EFFECTS IN THE WATER TREATMENT EXPERIMENT

The effect each variable and variable interaction has on metal ion removal in the water treatment experiment (Chapter 2) is a comparison of metal ion losses when the variable (or interaction of the variables) is used at its high (+) setting and its low (-) setting. The change in metal ion loss is the effect of the variable. The interaction effects are calculated from the experimental design table where, for example, interactions ABC for each experiment are found by multiplying A, B and C levels, the BCD interactions are the product of B, C and D signs, etc. (Table 16). The effects are calculated with equation 7:

$$\text{Effect of } X = \frac{2\sum X_i Y}{\sum X_i^2} \quad (\text{Equation 7})$$

where Y is the metal ion loss and  $X_i$  is the sign (+ or -) of the "i"th experiment for variable (or interaction) X. As an example of this calculation using variable A and copper data, the sum of the first eight copper losses ( $A_1$ - $A_8$  are all +) is subtracted from the sum of the last eight ( $A_9$ - $A_{16}$  are all -). This total is doubled and divided by 16 ( $\sum A_{1-16}^2$  where  $A_i$  is  $\pm 1$ ). The effect of variable A on copper losses is -1.38.

Table 16. Experimental Design for the Calculation of the Effects of Metal Ion Concentration, Alum Concentration, pH, Fulvic Acid Concentration and Interactions of These Variables on  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  Removal From Solution<sup>a</sup>

Exp. (i)	Variable Settings <sup>b</sup>															Metal Ion Losses (%)		
	A	B	C	D	AB	AC	AD	BC	BD	CD	ABC	ABD	ACD	BCD	ABCD	Cu	Cd	Zn
1.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	93	59	81
2.	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-	93	5	53
3.	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-	72	-5	-3
4.	+	+	-	-	+	-	-	-	-	+	-	-	+	+	+	23	-3	0
5.	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	7	0	-7
6.	+	-	+	-	-	+	-	-	+	-	-	+	-	+	+	54	2	16
7.	+	-	-	+	-	-	+	+	-	-	+	+	-	+	+	38	-6	-6
8.	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	13	-5	-4
9.	-	+	+	+	-	-	-	+	+	+	-	-	-	+	-	90	32	82
10.	-	+	+	-	-	-	+	+	-	-	-	+	+	-	+	96	14	49
11.	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	32	2	-1
12.	-	+	-	-	-	+	+	-	-	+	+	+	-	+	-	24	2	0
13.	-	-	+	+	+	-	-	-	-	+	+	+	-	-	+	6	3	-1
14.	-	-	+	-	+	-	+	-	+	-	+	-	+	+	-	64	8	21
15.	-	-	-	+	+	+	-	+	-	-	-	+	+	+	-	14	0	1
16.	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	24	-3	-2

<sup>a</sup>See the water treatment experiment in Chapter 2.

<sup>b</sup>Variables are A=metal ion concentration, B=alum concentration, C=pH, D=fulvic acid concentration.



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